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THE UNIVERSITY OF ALBERTA

ENHANCED ANAEROBIC BIOLOGICAL TREATMENT OF PHENOLIC WASTEWATERS

BY

WARREN B. KINDZIERSKI

©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ENHANCED ANAEROBIC BIOLOGICAL TREATMENT OF PHENOLIC WASTEWATERS submitted by WARREN B. KINDZIERSKI in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ENVIRONMENTAL ENGINEERING.

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Date Dec 15, 1988

ABSTRACT

The combined treatment requirements for a high strength phenolic wastewater were examined in batch and semicontinuous anaerobic methanogenic bioassays. Solvent extraction pretreatment and in-situ addition of activated carbon during anaerobic treatment were effective in removing phenol from a coal liquefaction wastewater from the H-coal process.

The selective pH adjustment of high strength phenolic wastewater followed by diisopropyl ether extraction reduced the phenolic concentration to non-inhibitory levels, and removed non-phenolic inhibitory compounds. The weakly acidic nature of phenol and substituted phenols allows for their selective removal by solvent extraction. Anaerobic bacteria were able to degrade phenol in the solvent extracted wastewater, however, the bacteria exhibited instability under semicontinous feeding conditions. The addition of activated carbon to the stressed phenol-degrading cultures improved their ability to remove phenol from solution.

Further investigation into the role activated carbon performed during anaerobic phenol treatment demonstrated its importance as a biological support, in addition to providing adsorptive capacity for organic (including inhibitory) compounds. The similar study of other support materials (ion exchange resins) which did not possess an adsorptive capacity for organic compounds supported these findings.

Excellent agreement was demonstrated among physical evaluation methods, performance bioassays, radiolabelled cell

adsorption studies, and scanning electron microscopy observations in judging the value of the materials as biological supports. The pore capacity and associated surface area were the most important features that encouraged biomass growth on the support materials. This successful evaluation allowed the development of criteria to assess the preference of a support material to provide optimum biomass development by characterizing the physical/chemical features that make an ideal biological support (pore capacity, surface area, substrate adsorptive capacity, and surface chemistry).

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LIST OF ABBREVIATIONS

A = pore cross-sectional area

AIC = average influent concentration

Alk. = alkalinity

a_w = solute activity in water

a_s = solute activity in an organic solvent

β = beta

BOD = biochemical oxygen demand

c = solute equilibrium concentration in solution

c_a = solute activity in water

c_e = adsorptive equilibrium concentration of solute

c_s = solute concentration in an organic solvent

C^* = apparent 2 hr equilibrium phenol concentration

C_o = solute influent concentration at time $t = 0$.

$C_{(o)}$ = aqueous phenol concentration at time $t = 0$.

COD = chemical oxygen demand

C_t = solute effluent concentration at time $t = t$.

$C_{(t)}$ = aqueous phenol concentration at time $t = t$.

D = distribution ratio

D = diffusion coefficient

DD = deionized distilled water

Dn = net diffusion coefficient of phenol through biomass and water

DTS = direct transmission spectroscopy

DIPE = diisopropyl ether

d_s = density of organic solvent

DVB = divinyl benzene

d_w = density of water

E_h = redox potential with respect to standard hydrogen electrode

ESCA = electron spectroscopy for chemical analysis

F = phenol flux

FTIR = Fourier transform infrared spectroscopy

¥ = gamma particle

¥$_s$ = activity coefficient of solute in an organic solvent

¥$_s^\infty$ = activity coefficient of solute in an organic solvent at infinite dilution

¥$_w$ = activity coefficient of solute in water

GAC = granular activated carbon

GC = gas chromatography

GS/MS = gas chromatography/mass spectrometry

G_s = free energy per mole of solute in organic solvent

G_s^o = standard free energy per mole of solute in organic solvent

G_w = free energy per mole of solute in water

G_w^o = standard free energy per mole of solute in water

HPLC = high pressure liquid chromatography

HRT = hydraulic retention time

IR = infrared

IX = ion exchange

k = Freundlich adsorption coefficient

k = reaction rate constant

K = equilibrium constant

K_a = acid equilibrium constant

K_c = equilibrium distribution constant (concentration units)

K_d = equilibrium distribution constant (weight fraction units)

K_x = equilibrium distribution constant (mole fraction units)

m.c. = micro-centrifuge

MIBK = methly isobutyl ketone

NAC = non-activated carbon

PAC = powdered activated carbon

PAS = photoacoustic spectroscopy

PVC = polyvinyl chloride

q = mass adsorbate per mass adsorbent at equilibrium

q^* = quasi-equilibrium mass loading based on a 2 hr contact time

R = Universal gas constant

RPM = revolutions per minute

s = solvent phase

SEM = scanning electron microscopy

T = absolute temperature (°K)

t = time period in days

t_d = hydraulic retention time in days

TOC = total organic carbon

VOA = volatile organic acids

VSS = volatile suspended solids

v/v = volume per volume

V = volume of reactor

v_s = molar volume of solvent

v_w = molar volume of water

w = water phase

W = mass of activated carbon

XPS = x-ray photoelectron spectroscopy

1.0 INTRODUCTION

Phenolic compounds are widely used in synthetic chemical processes. Phenol was among the 50 chemicals produced in greatest quantity in the United States in 1983 (Webber, 1984). Wastewaters from industrial operations that produce and use phenolic compounds as raw manufacturing materials will contain these compounds as pollutants. Likewise, phenolic compounds are pollutants in the wastewaters from coal conversion processes, petroleum refining, and coal coking processes. The environmental implications of aqueous phenol pollutants arise because of their potential to cause taste and odor, and toxicity problems in fish and other organisms that reside in natural water systems receiving phenolic wastewaters.

The treatment alternatives chosen for a phenolic wastewater are based on phenolic concentration, the presence of significant amounts of other organics (typically associated with such wastewaters), and the economic feasibility of the process. Treatments that have been shown to be effective include: oxidation, physical adsorption, solvent extraction, and biological methods (aerobic and anaerobic) (Wang et al., 1986).

The limitation (or disadvantage) of employing physical/chemical processes for phenolic wastewater treatment is their inability to economically produce an effluent of acceptable discharge quality. The operating costs rise dramatically as better effluent quality objectives are pursued. The limitation of the biological processes is their inability to treat high concentrations of phenolics associated with industrial wastewaters. The inhibition potential of phenol itself and the presence of other biological inhibitors (organic or inorganic) are a major drawback.

An alternative scheme employing a combination of physical, chemical, and biological processes may be a practical approach. The solution would be addressed in two stages:

i) Physical/chemical treatment to render the wastewater amenable to biological treatment (i.e. remove inhibitors), and
ii) Biological treatment to render the wastewater acceptable for discharge.

The choice of aerobic or anaerobic biological treatment for the removal of soluble organics (e.g. phenol) from industrial wastewaters warrants some discussion. While both processes have organisms capable of degrading phenolic compounds, distinct advantages of the anaerobic process are apparent:

i) Greater costs are associated with handling and disposal of aerobic biomass than anaerobic biomass. Aerobic bacteria typically produce 3 to 5 times the biomass produced anaerobically per unit of similar substrate removed.
ii) As less anaerobic biosolids are produced per unit of organic removed, the nutrient requirements (e.g. nitrogen and phosphorus) are lower compared with the aerobic process.
iii) Added energy costs are associated with supplying the oxygen required by the aerobic bacteria to degrade organic compounds.
iv) A major end product of the anaerobic process is methane (a recoverable fuel source).

A major limitation of the anaerobic process is related to its low biomass production rate. The lower biomass yield makes biomass retention important for the successful operation of the process. This becomes especially important during the treatment of industrial wastewaters that may contain organics which are difficult to biodegrade.

Current research in the environmental engineering and fermentation biotechnology fields focuses on methods for improving the biomass retention capabilities in biological processes. Providing some type of support material for microbial attachment or immobilizing the microorganisms in the process have resulted in enhanced performance compared to suspended-growth systems. This finding makes attached-growth systems well suited to anaerobic processes.

In the case of anaerobic treatment of phenolic (industrial) wastewaters, activated carbon has been successfully used as both a biological support and an organic adsorbent. However, little evidence of the mechanisms responsible for the enhanced performance of activated carbon as a biological support has been obtained.

This research will examine the treatment requirements for an authentic industrial phenolic (coal conversion) wastewater employing anaerobic biological treatment, and, attempt to focus on the mechanisms by which activated carbon acts to allow anaerobic degradation of phenol.

2.0 LITERATURE REVIEW AND RESEARCH OBJECTIVES

This chapter will review the anaerobic biodegradation of phenolic compounds, the treatment of phenolics in industrial wastewaters, and the association of microorganisms with support materials. Research objectives that address the anaerobic treatment of phenolic wastewaters will be presented.

2.1 Anaerobic Degradation of Phenolic Compounds

The anaerobic bioconversion of phenolic compounds to end products of methane, carbon dioxide, and new biomass is influenced by several components:

i) the aqueous environment,
ii) the microbial diversity, and
iii) the organic carbon source present.

The following discussion applies to engineered biological systems and not to natural aquatic environments (e.g. marshes, lake sediments). It is limited to the degradation of phenol and the three cresol isomers.

2.1.1 Environmental Conditions

The aqueous environment in which anaerobic microbes reside should be defined with respect to temperature, pH, redox, and available nutrients. All microorganisms exhibit maximum and minimum activities across a temperature range. The mesophilic organisms (temperature range 25-45°C) have an optimum activity temperature of 35-37°C in anaerobic biological systems (Stronach et al., 1986).

pH is a major factor governing anaerobic processes. Most microorganisms exhibit optimal activity between pH 6.5 and 7.5. The control of pH is important because microbial activity is inhibited below 5.0 and above 8.5. Maintenance of the pH is provided predominantly by the alkalinity/buffering influence of the carbonate system in the 6.0 to 7.7 range (Stronach et al., 1986). Many industrial wastewaters lack sufficient buffer capacity for the anaerobic processes and must be supplemented with some form of carbonate/bicarbonate alkalinity.

The redox potential (degree of oxidation or reduction) of the aqueous environment in which anaerobic bacteria operate is critical. The potential state is produced by the presence, or absence, of chemical oxidants (e.g. oxygen) or reductants (e.g. sulfide). Oxidants impart a high potential, while reductants impart a low potential (favorable to anaerobic bacteria). Methane formation requires a redox potential (E_h) of < -200 mV to proceed (Berry et al., 1987). Some anaerobic processes can be irreversibly inhibited by high potentials (i.e. $E_h >> -200$ mV) (Hungate, 1969). A combination of microbial processes is necessary to mediate a redox potential consistent for methanogenic activity. Microbial oxygen scavenging, and sulfide formation are required for the maintenance of a low redox potential in a diverse anaerobic population.

The nutritional requirements for anaerobic microorganisms are not generally met in industrial wastewaters. The macro-nutrients include carbon (C), nitrogen (N), and phosphorus (P). Although C is readily available in organic substrates for bacteria, N and P are usually not abundant and must be added to promote optimum biological activity. A study by Britz et al. (1988) examined the N and P requirements for the anaerobic treatment of petrochemical effluent. They found a minimum of 45 mg/L N (as Total Kjeldhal Nitrogen) and 8 mg/L (as PO_4-P) were sufficient to operate a downflow fixed-bed anaerobic process and maintain >95% removal of chemical oxygen demand (COD). Approximately 45% of the wastewater COD was acetate which can be directly converted to methane by methanogenic bacteria.

Fedorak and Hrudey (1986b) were able to show the omission of bicarbonate (HCO_3^-), major cation salts (Na^+, NH_4^+, Ca^{2+}, and Mg^{2+}), or phosphate was sufficient to impair methanogenic consortia from degrading phenol and p-cresol in draw and feed cultures. The failure of bicarbonate-deficient cultures was not attributed to a pH decrease which suggests that the microbial consortia required HCO_3^- (CO_2) for dissimilatory or assimilatory purposes.

Other elements and vitamins are also required in trace quantities to encourage optimal growth and activity. Table 2.1 summarizes the trace nutrients that have been added to or shown to be required by anaerobic bacteria for the treatment of complex soluble organics in industrial wastewaters.

2.1.2 **Microbial Diversity**

Anaerobic degradation of phenolic compounds requires a consortium of bacteria to accomplish a complex series of

Table 2.1 Trace Nutrient Requirements of Anaerobic Bacteria in Methanogenic Consortia

Nutrients	Reference
B, Cu, Mn	Speece and McCarty (1964)
B Vitamins	Owen et al. (1979)
V, Zn	Buggins (1981)
Co, Fe, Ni, S	Speece (1983)
Mo, Se, W	Stronach et al. (1986)

biochemical reactions. Four distinct microbial processes that might interact during phenolic degradation are represented in Table 2.2 (after Berry et al., 1987). The fermentative group of bacteria would be responsible for the initial conversion of phenol to short chain carboxylic acids. The sulfate-reducers and CO_2-reducing methanogens are the critical species in the overall process. The presence of either is required for scavenging H_2 and allowing proton reduction (ß oxidation) reactions to proceed. Either group must be present in sufficient numbers to keep the H_2 concentration low. The acetate-utilizing methanogens are also important for scavenging acetate to encourage ß oxidations (McCarty and Smith, 1986). Beta oxidation reactions are not thermodynamically favorable unless the pool of metabolic products (acetate and H_2) is sufficiently low.

Healy and Young (1978, 1979) have shown that relatively long lag times are evident prior to the onset of phenol degradation in unacclimated microbial consortia. A probable explanation is that the bacteria capable of degrading phenol (i.e. the fermentative species, Table 2.2) are initially present in low numbers and the lag period represents the time required for the species to increase in numbers. Examples of microorganisms representing the different metabolic processes in Table 2.2 are presented in Table 2.3.

Dwyer et al. (1986) proposed from microorganism enrichment studies that a consortium of fermentation and methanogenic species were required to degrade phenol to CH_4 and CO_2. They tentatively identified a phenol-oxidizing bacterium (capable of fermentation and ß oxidation), and two species of methanogens (H_2-utilizing methanogen and an acetate-utilizing methanogen). In theory, all

Table 2.2 Anaerobic Microbial Processes
(After Berry et al., 1987)

PROCESS	REACTION
1. Fermentation	OM ====> carboxylic acids (acetate, propionate, or butyrate)
2. Dissimilatory sulfate reduction	OM (or H_2) + $SO_4^=$ ====> H_2S + CO_2
3. Proton reduction (Beta oxidation)	OM (C_4 - C_8 CA) + H^+ ====> H_2 + acetate
4. Methanogenesis	CO_2 + H_2 ====> CH_4 acetate ====> CH_4 + CO_2

OM = organic matter.
CA = carboxylic acid.

Table 2.3 Anaerobic Microbial Diversity for Phenol Degradation

SPECIES	SUBSTRATE	REFERENCE
1. Fermentation:		
unidentified	phenol	Dwyer et al. (1986)
2. Dissimilatory sulfate reduction:		
Desulfobacter postgatei	acetate	Postgate (1984)
3. Proton reduction:		
Syntrophobacter wolinii	propionate	Boone and Bryant (1980)
unidentified	butyrate + caproate	McInerney et al. (1979)
4. Methanogenesis:		
Methanobacterium sp.	$H_2 + CO_2$, formate	Mosey (1982)
Methanobrevibacter sp.	$H_2 + CO_2$	"
Methanococcus sp.	$H_2 + CO_2$, formate	"
Methanomicrobium sp.	$H_2 + CO_2$, formate	"
Methanogenium sp.	$H_2 + CO_2$, formate	"
Methanospirillum sp.	$H_2 + CO_2$, formate	"
Methanosarcina sp.	$H_2 + CO_2$, methanol, acetate	"
Methanothrix sp.	acetate	Huser et al. (1982)

requirements for the interspecies transfer of metabolic products (acetate and H_2) are satisfied with the consortium present.

2.1.3 Metabolic Fate of the Carbon Source

The metabolic fate of phenolics depends on the individual microbial species that predominate in an anaerobic mixed consortium. A summary of the proposed fate of p-cresol and phenol is provided in Figure 2.1 (after Senior and Balba, 1984; Young and Rivera, 1985). It is presumed that the aromatic ring is reduced prior to cleavage and formation of fatty acids. Knoll and Winter (1987) provided evidence that benzoate may be an intermediate in the metabolic process, however the mechanism by which benzoate formed was unknown. Roberts et al. (1986) demonstrated that most of the methyl carbon from p-cresol (92%) was oxidized to CO_2 (HCO_3^-) while 87% of the methyl carbon of m-cresol was converted to CH_4 in a methanogenic consortia.

In contrast to the fates of m- and p-cresol, o-cresol has not always been shown to be biologically degraded under anaerobic conditions. Suidan et al. (1981a), Boyd et al. (1983), and Fedorak and Hrudey (1984) did not observe o-cresol degradation under anaerobic conditions. However, Earley (1988) was able to observe o-cresol degradation to methane in batch anaerobic cultures. Earley used phenol acclimated bacteria in anaerobic toxicity assays (ATA - Owen et al., 1979) and tested the potential of o-cresol to impair phenol degradation. He found that the ATA cultures were able to degrade o-cresol 100 days after they had degraded phenol. The bacteria had

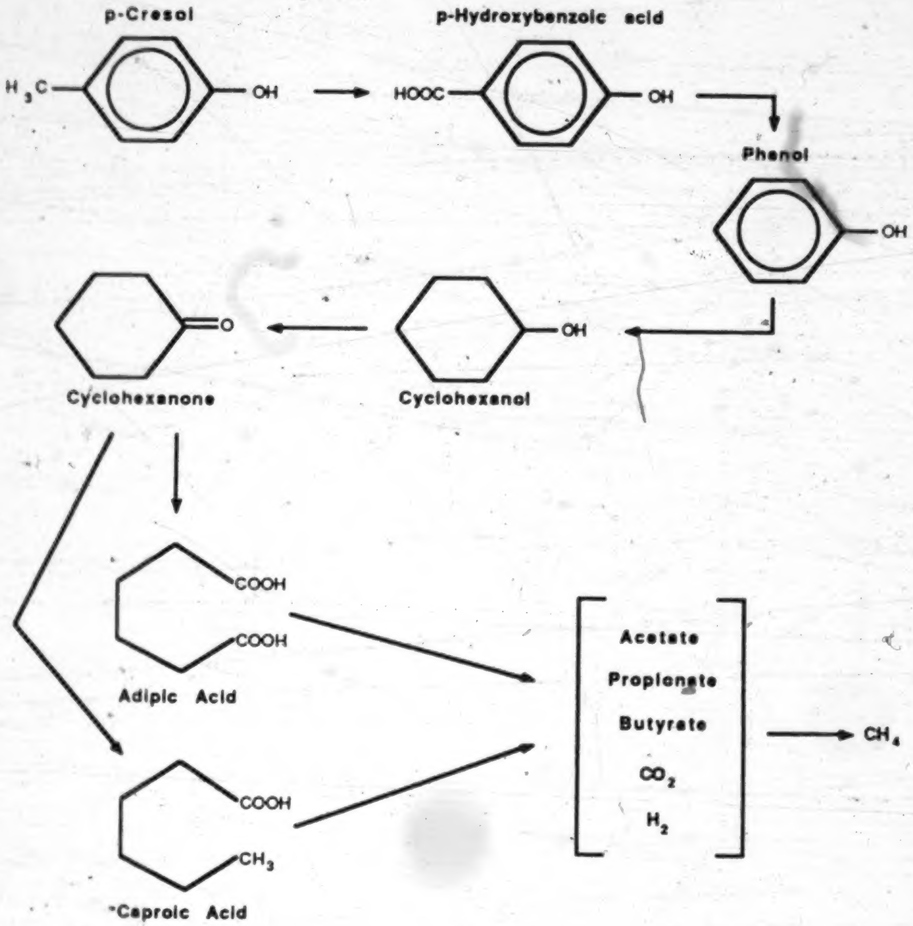
p-Cresol
H_3C
OH
p-Hydroxybenzoic acid
HOOC
OH
Phenol
OH
Cyclohexanone
O
Cyclohexanol
OH
COOH
COOH
Adipic Acid
COOH
CH_3
Caproic Acid
Acetate
Propionate
Butyrate
CO_2
H_2
CH_4

Figure 2.1 Fate of p-Cresol and Phenol under Anaerobic, Methanogenic Conditions (Adapted from Senior and Balba, 1984; and Young and Rivera,1985)

been acclimated to pure phenol for one year prior to the ATA study. A possible explanation for the observed difficulty of o-cresol degradation may be seen in its structure. The presence of hydroxyl (-OH) attached to the aromatic ring changes the electron distribution in the ring near the hydroxyl position. This affects the ability of microbial enzymes to attack the methyl carbon in the ortho position. If only certain microbial enzymes are capable of attacking the methyl carbon in the ortho position, the non-biodegradability of o-cresol reported in some studies may be explained by the absence of the organisms that produce these enzymes.

2.2 Phenolic Wastewater Treatment Technology

Phenolic wastewaters from industrial processes can contain high concentrations of phenolics and a wide variety of other organics. Liquid and gaseous coal conversion are two processes that generate wastewaters with these characteristics.

2.2.1 Coal Conversion Processes and Wastewater Generation

The goal of coal conversion processes, both liquefaction and gasification, is to increase the hydrogen-to-carbon (H/C) mass ratio of coal to produce a liquid or gaseous fuel and/or products that could be further upgraded to desired fuels (Senetar and King, 1986). There are three principal ways to liquefy coal (Moschopedis and Hepler, 1987):

i) break the coal down to gaseous products and reassemble these into liquid products,

ii) pyrolysis - remove carbon from the coal by thermal treatment, and

iii) direct hydroliquefaction - incorporate hydrogen into the chemical structure of the coal.

Coal gasification involves pyrolysis in the presence of air (or oxygen) and high temperature steam (Senetar and King, 1986). Steam provides a necessary source of hydrogen to increase the H/C ratio of the product.

Both processes give rise to a wide variety of organic compounds (some of high concentration) in their wastewaters. The wastewaters arise from product washing and steam stripping applications. Giabbai et al. (1985) characterized the major organic pollutants from a coal gasification process (Table 2.4). Phenol and the mono-methyl phenols (cresols) are known to be the dominant organics in the wastewaters from both processes. Senetar and King (1986) indicate that the extent of phenol formation is reduced at higher coal gasification temperatures. Less phenol would be present in the wastewaters from this technology.

Table 2.5 provides a partial analysis of a coal liquefaction wastewater from the direct hydroliquefaction "H-coal" pilot plant process at Catlettsburg, Kentucky (Fedorak and Hrudey, 1986a). Phenol constitutes the dominant organic in the wastewater, accounting for 55% of the COD. The sample was steam stripped prior to the analysis, which accounts for the low ammonia-nitrogen content (6.4 mg/L).

Table 2.4 Major Organic Compounds Identified in Coal Gasification Wastewater (After Giabbai et al., 1985)

Acid Fraction	Neutral Fraction	Base Fraction
phenol	benzene	pyridine
o-cresol	toluene	2-picoline
m-, p-cresol	cyclopentanone	3-picoline
2,4-dimethylphenol	ethylbenzene	4-picoline
2,5-dimethylphenol	o-xylene	C_2-pyridine
3,5-dimethylphenol	m-, p-xylene	C_1-aniline
2,3-dimethylphenol	methoxybenzene	C_2-aniline
3,4-dimethylphenol	benzonitrile	quinoline
3-ethylphenol	naphthalene	isoquinoline
4-ethylphenol	indole	
dimethoxybenzene	acenaphthalene	
naphthol	dibenzofuran	
	fluorene	
	phenanthrene	
	anthracene	
	pyrene	

Table 2.5 Analysis of H-coal Liquefaction Wastewater[1] (After Fedorak and Hrudey, 1986a)

Parameter	Concentration (mg/L)
pH	7.4
Organic carbon	7,600
COD	21,100
Total Kjeldhal nitrogen	267
Nitrite nitrogen	0.2
Nitrate nitrogen	0.8
Ammonia nitrogen	6.4
Total cyanide	0.21
Total phosphorus	5
Phenolics:	
Phenol	4,900
o-Cresol	586
m-Cresol	1,230
p-Cresol	420
2,4/2,5-Dimethylphenol	63
3,5-Dimethylphenol	213
3,4-Dimethylphenol	44
Volatile organic acids:	
Acetic acid	280
Propionic acid	160
n-Butyric acid	50
n-Valeric acid	35

---

[1] From the 544 metric tonne/day H-coal pilot plant located at Catlettsburg, Kentucky.

The treatment alternatives for phenolic industrial wastewaters present a considerable challenge and their success of treatment has been mixed.

2.2.2 Treatment Processes for High Strength Phenolic Wastewaters

The treatment technology employed for high strength phenolic wastewaters (including coal conversion wastewaters) involves physical, chemical, and biological (anaerobic and aerobic) methods. Studies have been conducted using both synthetically prepared and authentic wastewaters. A brief review of the technology will be presented. The objective of the review is to single out technologies that may be compatible to biological processes (specifically anaerobic), and to assess the effectiveness of the biological processes.

2.2.2.1 Physical/Chemical Processes

Several authors have investigated the viability of physical/chemical processes as partial treatment for phenolic wastewaters. A summary of the alternatives is listed in Table 2.6.

Solvent extraction is a popular alternative for coal conversion wastewaters because of its effectiveness for removal of phenolic compounds. These compounds generally comprise 40 to 80% of the COD fraction and are present at high concentrations. The remaining organic components consist of a wide variety of compounds which may also be removed by

Table 2.6 Physical/Chemical Methods for the Removal of Organics in Phenolic Industrial Wastewater

Process	Selected References
Solvent extraction	Greminger et al. (1982) Medzadourian et al. (1983) Senetar and King (1986)
Adsorption	Senetar and King (1986)
Stripping	Senetar and King (1986)
Wet air oxidation	Drummond et al. (1985)

solvent extraction. The desirable qualities of solvent extraction include:

i) high capacity for phenolics removal,

ii) capacity for removal of other background organics that are normally associated with the wastewater,

iii) relatively low aqueous solvent solubility, and

iv) potential for the recovery of both solvent and phenol.

Table 2.7 shows the boiling points of selected solvents and the dominant phenolics in coal conversion wastewaters. The large differences between the solvent and solute (phenolic) boiling points allow a process such as distillation to be effective for solvent recovery. This makes solvent extraction with solvent recovery an attractive treatment alternative. Extraction studies performed by Greminger et al. (1982), Medzadourian et al. (1983), and Senetar and King (1986) focused on the adequacy for phenol removal from pure water, and demonstrated the ability to easily concentrate phenol into the solvent phase. Further studies of solvent extraction of actual wastewaters (in combination with biological treatment) are discussed in Section 2.2.2.3.

Senetar and King (1986) also investigated adsorption (activated carbon and synthetic XAD resin) and stripping (nitrogen gas) for organics removal. Stripping was shown to be ineffective for phenolics removal because of the extremely low volatilities of phenolic compounds.

Drummond et al. (1985) were able to demonstrate that wet air oxidation could remove 100% of the phenol (9,150 mg/L)

Table 2.7 Boiling Points of Low-Boiling Solvents and Selected Industrial Wastewater Phenolics (After Senetar and King, 1986)

Solvent/Solute	Boiling Point (°C)
Low boiling solvents:	
Diisopropyl ether (DIPE)	68.3
Butyl acetate	125
Methyl isobutyl ketone (MIBK)	116.8
Selected Phenolics:	
Phenol	181.4
o-Cresol	190.8
m-Cresol	202.8
p-Cresol	202

and 90% of the COD in an untreated coal liquefaction wastewater (total COD 51,900 mg/L). However, further treatment would be necessary to remove the remaining COD.

2.2.2.2 **Anaerobic Biological Processes**

Anaerobic biological treatment studies of high strength phenolic wastewater have focused on phenolics removal. A summary of the anaerobic studies is provided in Table 2.8.

Fedorak and Hrudey (1984) investigated batch anaerobic treatment of phenol and various mono- and di-methyl phenols with municipal digestor bacteria unacclimated to phenolics. Only phenol (≤500 mg/L) and p-cresol (≤400 mg/L) were fermented to methane in 45-day experiments. Similar studies by Blum et al. (1986) were conducted using unacclimated and acclimated bacteria. They found that, in general, the presence of phenol acclimated bacteria did not improve the ability of the cultures to subsequently degrade other phenolics. All phenolics were found to inhibit cultures at high enough concentrations, and, Fedorak and Hrudey (1984) attributed the reduced performance to inhibition of the phenolic-degrading organisms.

Fedorak and Hrudey (1985, 1986a) studied batch and semicontinuously fed anaerobic treatment of diluted authentic coal liquefaction wastewater. They found that dilution of the wastewater to non-inhibitory phenolic levels did not remove the inhibition potential. They were able to associate this inhibition to non-phenolic ether-extractable substances. m-

Table 2.8 Anaerobic Biological Treatment of Phenolic Industrial Wastewaters

Wastewater	Selected References
Phenolics[1,2]	Fedorak and Hrudey (1984)
Coal liquefaction[1,3]	Fedorak and Hrudey (1985)
Coal liquefaction[1,3]	Fedorak and Hrudey (1986a)
Coal conversion[1,2,4]	Blum et al. (1986)
With Activated Carbon:	
Catechol[2,4]	Suidan et al. (1980)
Phenol [2,4]	Khan et al. (1981)
Phenol [2,4]	Suidan et al. (1981a)
Phenol [2,4]	Suidan et al. (1981b)
Aircraft paint stripping[3,4]	Khan et al. (1982)
Coal gasification[3,4]	Cross et al. (1982)
Coal gasification[2,4]	Suidan et al. (1983a)
Coal gasification[3,4]	Harper et al. (1983)
Coal gasifiaction[3,4]	Suidan et al. (1983b)
Coal gasification[2,4]	Wang et al. (1984)
Coal liquefaction[1,3]	Fedorak and Hrudey (1985)
Coal gasification[3,4]	Pfeffer and Suidan (1985)
Phenol[2,4]	Wang et al. (1986)
Phenol[2,4]	Kim et al. (1986)
Coke oven waste[3,4]	Edeline et al. (1986)
Coal gasification[2,4]	Suidan et al. (1987)
Coal coking waste[1,3]	Fedorak and Hrudey (1987)
Coal gasification[3,4]	Fox et al. (1988)
Refinery sour water[3,4]	Gardner et al. (1988)

[1] Serum bottle study.
[2] Synthetically prepared wastewater.
[3] Authentic wastewater.
[4] Laboratory reactor study.

Cresol was found to be biodegraded, however the microorganisms responsible for degrading this compound encountered difficulties establishing themselves in the semicontinuous cultures. When initially tested as a single substrate with unacclimated batch cultures (45-day incubation), m-cresol was not biodegraded (Fedorak and Hrudey, 1984).

Blum et al. (1986) operated phenol acclimated anaerobic filters (gravel packed) fed various synthetic phenols. They observed 100% biological removal of phenol (1,885 mg/L average influent concentration - AIC) in one filter. The gravel packed filter provided full conversion of phenol to methane. The authors also observed partial removal of 3,4-dimethylphenol (25% removal - AIC 55 mg/L) and o-cresol (78% removal - AIC 116 mg/L) in separate filters. However, no methane production was observed in both filters which suggests methane bacteria did not take part in the removal process.

Studies with Activated Carbon

Suidan et al. (1980) employed granular activated carbon (GAC) - anaerobic packed reactors for the successful treatment of a synthetic catechol (1,2-dihydroxybenzene) wastewater at 1,000 mg/L. The GAC was able to develop an acclimated catechol-degrading culture only after a long lag period (150 days). Prior to this, catechol was being removed from the wastewater by GAC adsorption. Suidan et al. (1981a, 1981b) studied synthetically prepared wastewaters containing phenol, o-cresol, and catechol using the same system. Only o-cresol was not degraded after biological activity became established on the GAC reactor.

Khan et al. (1981) treated phenol concentrations as high as 1,000 mg/L in three-stage GAC packed-bed reactors and achieved 92% removal. However, as mentioned previously, Blum et al. (1986) were able to achieve 100% phenol removal at higher concentrations (1,885 mg/L) using gravel packed anaerobic filters. The GAC used by Khan et al. (1981) did not provide any obvious benefits for phenol removal when compared to the study by Blum et al. (1986). However, Khan et al. (1982) were able to demonstrate the use of GAC was superior to anthracite in anaerobic packed-bed treatment of diluted aircraft paint stripping wastewater (containing 500 mg/L phenol). The GAC reactor (82% phenol removal) was superior to the anthracite reactor (54% phenol removal) under steady-state conditions. Methane production rates from the GAC reactor (0.67 L/day) and the anthracite reactor (0.36 L/day) indicate that increased methanogenic activity was responsible, in part, for the improved performance of the GAC reactor.

Cross et al. (1982) employed two anaerobic packed-bed reactors in series (plastic Rashig ring followed by GAC) for the treatment of an authentic coal gasification wastewater. The wastewater (26,900 mg/L COD) contained 5,600 mg/L phenol and was diluted 10-fold with water (i.e. 10% dilution) prior to being fed to the reactors. Phenol and cresol removals of 90 and 99% respectively were observed across the reactor system. However, only ≈10-20% of phenol removal and ≈58% cresol removal occurred in the plastic media packed reactor. The majority of methane (86%) was produced from the GAC reactor. Methanogenic activity was more established in the GAC reactor.

Suidan et al. (1983a) operated the same system as Cross et al. (1982) to study a synthetically-prepared coal gasification wastewater. The GAC was again superior to the plastic media during steady-state treatment of the synthetic wastewater. The first stage plastic filter only removed 11% of the influent COD while the GAC reactor achieved 78% removal of the influent COD. Suidan et al. (1983a) withdrew activated carbon from the GAC filter during the steady-state operating period for analysis. The GAC was extracted with ether, and the extract was analyzed by gas chromatography/mass spectrometry (GC/MS) in order to identify adsorbed organics. The authors identified many of the organics originally present in the feed, plus a host of other organics which were concluded to be biologically mediated by-products of the feed compounds. The COD equivalent of the methane produced in the GAC reactor accounted for only 50% of the influent COD, while 90% was removed across the reactor. A significant amount of COD was adsorbed on the GAC and this raises questions about the design life of the GAC adsorption capacity.

Suidan et al. (1983b) employed the same system as Cross et al. (1982) to study a 10% diluted coal gasification wastewater. The first stage plastic ring-packed reactor was providing very little organic removal and was taken out of service after 118 days of operation. The GAC-packed reactor established methanogenic activity after 30 days operation and remained active until the study was terminated (302 days). Again, the COD equivalent of the methane produced in the GAC reactor accounted for only 50% of the influent COD, while 85% was removed across the reactor. GC/MS examination of the ether extractable organics adsorbed on GAC media taken from the reactor after 302 days operation is shown in Table 2.9. The cresols and dimethylphenols accounted for >97% of the mass of extracted organics identified by GC/MS. The authors suggested cresols were refractory or slowly degraded under the conditions maintained in the GAC reactor. This may have accounted for their significant presence on the GAC, however eventual breakthrough of these compounds will occur once the GAC adsorption capacity is exceeded.

Harper et al. (1983) operated GAC anaerobic fluidized-bed reactors for the treatment of a 10-12% diluted coal gasification wastewater. They found that fresh addition of GAC was required for long term operating stability of the biological process. The GAC addition was necessary to continuously remove (by adsorption) organic inhibitory compounds that would otherwise accumulate in the reactor once the adsorptive capacity was spent. Likewise, it was

Table 2.9 GC/MS Identification of Ether-extracted Compounds from Activated Carbon During Anaerobic GAC Treatment of Coal Gasification Wastewater (After Suidan et al., 1983b)

Compound	Mass Extracted (mg/g)
Aniline	0.29
Phenol	0.15
o-Cresol	15.8
m/p-Cresol	42.3
o-Ethylphenol	0.72
2,4-Dimethylphenol	2.08
3,5- or 2,3-Dimethylphenol	9.28
3,4-Dimethylphenol	1.53
2,3,5-Trimethylphenol	0.75

necessary to remove some of the spent GAC consistent with the amount of fresh GAC added. While this technique effectively prolongs the adsorptive capabilities of the system, it provides unwanted removal (or loss) of anaerobic biomass associated with the spent GAC media. This loss reduces the biological capabilities of the system because anaerobic bacteria are generally slow growing.

Wang et al. (1984) operated a GAC anaerobic fluidized-bed reactor treating a synthetic feed of indole, quinoline, and methylquinoline (compounds previously identified in coal gasification wastewater). A six-month acclimation period was required for the reactor to develop a culture capable of degrading the polycyclic aromatic compounds to methane. The compounds were being removed by GAC adsorption during this period. The authors later suggested that only indole and quinoline were degraded to methane. Methylquinoline was the first compound observed to breakthrough the reactor in significant concentrations and cause biological failure of the reactor. Physical adsorption was the primary removal mechanism for this compound until saturation of the GAC and subsequent breakthrough. After operation of the reactor for 881 days the biological methane accounted for only 52% of the influent COD, while 92% of the influent COD was removed across the reactor (similar to what was observed by Suidan et al., 1983). The authors concluded that continuous and undisturbed biological operation of the system would require partial GAC removal and replacement. The GAC replacement is necessary for the continuous removal of adsorbable, refractory, and biological inhibitory compounds.

Fedorak et al. (1985) were able to demonstrate that the presence of activated carbon reduced the biological inhibition of a diluted coal liquefaction wastewater and allowed batch fermentation of phenolics. The authors tested various volume/volume (v/v) dilutions of the wastewater in the presence of different doses of activated carbon. Figure 2.2 shows the methane production from selected cultures after 80 days incubation at 37°C. The cultures with 4, 8, and 12% v/v dilutions of the H-coal liquefaction wastewater had phenolic levels of 304, 608, and 912 mg/L respectively. The activated carbon reduced the inhibitory nature of the wastewater at the higher v/v dilutions. The authors were able to demonstrate activated carbon reduced the time required by bacteria to acclimate to the wastewater by adsorbing non-phenolic inhibitors and allowing quicker adaptation to fermentable phenolics. Cultures from the 8% v/v H-coal dilution containing 1,000 mg/L activated carbon required 31 days before enhanced methane production occurred, whereas 59 days were required for the 500 mg/L activated carbon culture to show enhanced methane production.

Pfeffer and Suidan (1985) employed the same system as Wang et al. (1983) for the treatment of undiluted coal gasification wastewater (4,460 mg/L total phenolics). In view of what was observed by Wang et al. (1984), Suidan et al. (1983a, 1983b), and Harper et al (1983), a decision to replace

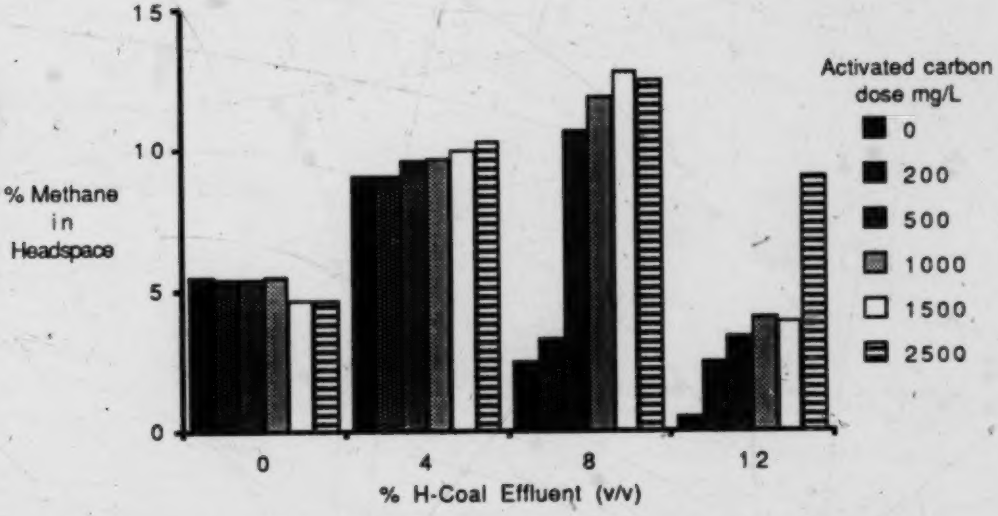

Figure 2.2 Methane Concentrations in Selected Cultures Containing Various Concentrations of H-coal Liquefaction Wastewater and Activated Carbon (Adapted from Fedorak et al., 1985)

the GAC at a rate of 10% (by reactor volume) per week was arbitrarily chosen. Excellent phenolics removal was observed under steady-state operation (300 days). A consequence of the carbon cycling procedure was that bacteria had a nominal solids retention time of 10 weeks. Biological activity no longer played the dominant role for phenolic removal under this protocol. Biological methane only accounted for 42% of the influent COD, while 94% was removed across the reactor during the study.

Wang et al. (1986) operated a GAC anaerobic fluidized-bed reactor to treat a synthetically-prepared phenol wastewater. They were able to demonstrate biological utilization was the major mechanism for >99% phenol removal (influent concentration 2,960 mg/L) once the microorganisms became established on the GAC media. The synthetic wastewater did not contain any of the background non-biodegradable or inhibitory organics that are typical of authentic phenolic industrial wastewaters (e.g. coal gasification wastewater - Table 2.4). As a result, the phenolic-degrading consortium became well established in the reactor.

Kim et al. (1986) used two stage GAC anaerobic expanded-bed reactors to treat a synthetic phenol wastewater. The authors suggested that phenol previously adsorbed on GAC could be desorbed and biodegraded (i.e. bioregeneration) to methane when the influent phenol concentration was reduced. Increased biological gas production rates had occurred during a period when influent phenol levels were lowered.

Edeline et al. (1986) examined the anaerobic treatment of a coke plant (phenolic) wastewater. The authors used a two-stage system employing an activated carbon fluidized-bed, followed by a polyurethane foam fixed-bed reactor. The system successfully treated steady-state influent phenol concentrations of 1,250 mg/L in the presence of 400 mg/L thiocyanate. Previous operation of the anaerobic process without activated carbon met with a series of failures. Inhibition by thiocyanate and phenol was responsible for the failures. The two-stage system provided good retention of the bacteria and a sink for inhibitors. The authors agreed that activated carbon replacement would be necessary to provide extended adsorptive removal of the inhibitors. The system did not remove the thiocyanate.

Suidan et al. (1987) and Fox et al. (1988) employed two anaerobic reactors in series (packed-bed plastic media and expanded-bed GAC) for the treatment of synthetically-prepared coal gasification wastewater. The wastewater contained high concentrations of compounds which the authors reported as inhibitory. The plastic media reactor provided very little reduction of wastewater organics. The GAC reactor was able to achieve excellent organics removal provided the GAC media was periodically replaced.

Fedorak and Hrudey (1987) studied the batch anaerobic degradation of phenolics in dilutions of coal coking wastewater. The authors investigated prior neutral pH ether extraction and in-situ activated carbon treatment of the wastewater. The neutralized coke effluent was extracted seven times with diethyl ether at an effluent:solvent ratio of 6.67:1. The in-situ activated carbon addition was 2,500 mg/L. Activated carbon was found to be more effective for relieving the inhibition encountered in unextracted coke effluent than that encountered in the ether-extracted coke effluent (Table 2.10). The cultures supplemented with activated carbon required shorter acclimation times to produce methane in excess of the control culture. However, neither treatment completely relieved the inhibition as methane yields were still lower than expected for complete phenol removal from both treatments. Cyanide, initially present at 8.3 mg/L in the wastewater, was a suspected inhibitor.

Gardner et al. (1988) investigated GAC fluidized-bed anaerobic treatment of sour water stripper bottoms (phenolic levels ≈300-450 mg/L). Three fluidized-bed reactors were operated. Two were loaded with different sized GAC, and the other was loaded with non-activated carbon (NAC). The authors found that the NAC reactor performed poorly and failed long before the GAC reactors. o-Cresol was present in the supernatant from the NAC reactor prior to failure. The authors suspected that the negligible organic adsorption capacity of the NAC media was responsible for the failure by allowing organic inhibitors to accumulate in the reactor. The reactor charged with smaller sized GAC (83% COD removal) performed better than the large GAC media reactor (67% COD removal) under the highest organic loading conditions. The authors

Table 2.10 Acclimation Times of Selected Batch Cultures Receiving Various Dilutions of Coke Effluent or Ether-extracted Coke Effluent (After Fedorak and Hrudey, 1987)

	Acclimation Time (days)[1]	
Dilution[2]	Coke Effluent[3]	Ether-extracted Coke Effluent[4]
10%	17	15
20%	17	20
30%	22	24
40%	24	>43
50%	29	>43

[1] Acclimation time at which the mean methane concentration in cultures supplemented with 200 mg/L phenol exceeded that of control culture (which did not contain any phenol, coke effluent, or ether-extracted coke effluent).

[2] % (by volume) concentration of coke effluent or ether-extracted coke effluent.

[3] Contained 2,500 mg/L activated carbon.

[4] Solvent extracted with diethyl ether seven times at a wastewater to solvent ratio of 6.67:1.

speculated that the increased surface area for microbial attachment and decreased diffusional resistance to adsorption of the smaller sized GAC media was responsible for the improved performance. The authors did not mention what portion of the total surface area was available for microbial attachment. Most of the surface area of GAC is associated with pores that may be of a size too small for microorganisms to enter.

2.2.2.3 Combined Physical Chemical Biological Processes

Several studies have focused on the complete treatment requirements for coal conversion wastewaters. Table 2.11 lists the unit processes investigated in some of the studies.

Luthy et al. (1983) examined coal gasification wastewater treatment employing solvent extraction, steam stripping, (aerobic) activated sludge with and without powdered activated carbon (PAC), lime-soda softening, and GAC adsorption. Stepwise performance of the unit processes are demonstrated in Table 2.12. Solvent extraction readily removed >99% of the phenolics. However, significant levels of non-extractable organics still remained (i.e. COD 3,900 mg/L) which required further treatment. Steam stripping successfully reduced ammonia-nitrogen concentrations from 4,400 to 30 mg/L. Aerobic biological treatment and GAC adsorption was used to remove the non-extractable organics.

Table 2.11 Physical/Chemical/Biological Treatment of Coal Conversion Wastewater

Wastewater	Unit Process - Component Removed	Selected Reference
Coal Gasification	Solvent extraction - phenolics Steam stripping - ammonia, dissolved gases Activated sludge/PAC - organics Lime soda softening - hardness GAC adsorption - organics	Luthy et al. (1983)
Coal Liquefaction	Stripping - ammonia Solvent extraction - phenolics Nitrogen stripping - solvent Activated sludge - organics GAC adsorption - organics	Drummond et al. (1985)
Coal Gasification	Filtration - residual tar Solvent extraction - phenolics Nitrogen stripping - solvent Steam stripping - ammonia, dissolved gases Activated sludge - organics Filtration - biological solids GAC adsorption - organics	Gallagher and Mayer (1985)
Coal Gasification	Solvent extraction - phenolics Hot gas stripping - ammonia Activated sludge/PAC - organics	Humenick and Shellenbarger (1986)

PAC = powdered activated carbon.
GAC = granular activated carbon.

Table 2.12 Treatment Characteristics of Coal Gasification Wastewater (mg/L) (After Luthy et al., 1983)

Parameter	Raw Wastewater	After Solvent Extraction[1]	After Stripping	After AS[2]	After GAC[3]
TOC	11,100	1,950	1,380	580	125
COD	32,000	3,900	2,980	1,340	315
BOD	26,000	2,900	1,820	32	0
Phenolics	5,500	5	3	0.1	0
Org.-N	115	51	33	10	1.7
NH_3-N	6,300	4,400	30	84	88
SCN^-	120	110	105	4	1.3
S^{2-}	100	75	< 10	0	0
Total S (as S)	380	156	0	0	0
Ca^{2+} (as $CaCO_3$)	0	10	740	N.D.	N.D.
Alk. (as $CaCO_3$)	20,700	16,300	850	175	N.D.

[1] Solvent used was methyl isobutyl ketone.

[2] Activated sludge without powdered activated carbon.

[3] Granular activated carbon.

N.D. = Not determined.

Drummond et al. (1985) employed stripping, solvent extraction, activated sludge treatment, and GAC adsorption for a coal liquefaction wastewater from the H-coal process. Stepwise unit performance of the system is provided in Table 2.13. Stripping and solvent extraction reduced ammonia-nitrogen levels from 5,500 to 160 mg/L and phenolic levels from 9,150 to 40 mg/L. Sulfides were adequately removed by stripping. Further treatment was necessary to reduce residual organics (COD 2,220 mg/L).

Studies performed by Gallagher and Meyer (1985) and Humenick and Shellenbarger (1986) showed findings similar to Luthy et al. (1983). The former two studies were able to demonstrate the utility of using solvent extraction as an effective pretreatment to a biological process. However, stripping of the wastewater was generally required after extraction to keep the residual solvent concentration low prior to activated sludge treatment.

2.2.3 Discussion of Treatment Technology

Phenolic wastewaters (e.g. coal conversion wastewaters) contain high concentrations of phenolics and a wide variety of other organics. Some of the other organics are non-biodegradable and inhibitory to biological processes at the concentration they are present in the wastewaters. In addition, phenol is inhibitory to microorganisms at the elevated concentrations found in some wastewaters.

Table 2.13 Treatment Characteristics of Coal Liquefaction Wastewater (mg/L)
(After Drummond et al., 1985)

Parameter	Raw Wastewater	After Stripping	After Solvent Extraction[1]	After AS[2]	After GAC[3]
TOC	11,030	10,390	725	210	5
COD	51,900	34,600	2,220	550	15
BOD	15,900	0	950	5	0
Phenolics	9,150	8,560	40	< 1	< 1
NH_3-N	5,500	1,000	160	110	100
SCN^-	15	10	< 5	< 5	< 5
S^{2-}	3,900	< 10	< 10	< 10	< 10

[1] Stripped of residual solvent (methyl isobutyl ketone).

[2] Activated sludge

[3] Granular activated carbon.

Numerous authors have employed granular activated carbon in anaerobic biological processes. The activated carbon was intended to serve as a biological support for microorganisms and/or as an adsorbent for the removal of organic inhibitors. In fact, the adsorptive capability of activated carbon was necessary for inhibitory organic compound removal (in order to allow biodegradation of phenolic compounds to methane).

Many of the studies with synthetically-prepared phenol solutions demonstrated that anaerobic biological degradation was the dominant COD removal mechanism. Whereas, with authentic wastewaters both activated carbon adsorption and anaerobic biodegradation were required for COD removal. The main difference between the synthetic and authentic phenolic wastewaters is their background matrix. The absence of this background matrix (which includes a wide variety of organics typically associated with industrial wastewaters) in the synthetic wastes does not allow a good evaluation of the true performance of the anaerobic microorganisms. Many of the background organics are suspected biological inhibitors and require the presence of activated carbon to remove them. Fedorak et al. (1985) and Fedorak and Hrudey (1987) demonstrated that activated carbon could reduce this inhibition to allow anaerobic fermentation of phenolics in coal liquefaction and coal coking wastewaters.

Fox et al. (1988), Gardner et al. (1988), and Suidan et al. (1987) promoted the use of GAC for extended anaerobic treatment of phenolic wastewaters providing regular GAC replacement was practiced. They felt that in order to maintain the biological activity it was necessary to continuously cycle fresh carbon into the reactor to extend the adsorptive removal of organic inhibitors. A disadvantage of this procedure is that biological solids are continuously cycled out of the system. This reduces the biomass available for removal of organics. As mentioned previously, a limitation of the anaerobic process is related to its low biomass production rate. The lower yield makes biomass retention an important factor in the successful operation of the process. Other disadvantages of the regular carbon replacement option are the additional capital and operating costs associated with providing virgin or regenerated carbon to the system.

Many authors were able to prove the utility of using a biological process to treat phenolic wastewaters, however the inhibition potential of the wastewater had to be eliminated prior to biological treatment. Both phenol and non-phenolic compounds were associated with the inhibition. One effective inhibition control technique was solvent extraction. Research by Drummond et al. (1985) demonstrated that phenolic levels as high as 8,560 mg/L could be reduced to 40 mg/L by solvent extraction (Table 2.13). However, the adequacy of solvent extraction alone for treatment of phenolic wastewaters is questionable. Although the majority of phenolics and COD could be removed by extraction, residual organics would still persist in the effluent (non-extractable organics such as volatile organic acids and difficult to extract organics, e.g. dihydroxybenzenes). Many of these residual organics are degradable, and could very well be removed by a biological process. Residual COD levels of 2,220 mg/L persisted in the extracted effluent during the work by Drummond et al. (1985). Aerobic biological treatment was successful in removing 75% of the residual organics.

A majority of organics in phenolic industrial wastewaters are biodegradable by anaerobic microorganisms. Coal conversion wastewaters contain 40-80% of the COD as biodegradable phenolics. However, many residual organics are suspected biological inhibitors. Employing solvent extraction followed by an anaerobic biological process to treat these wastewaters may prove attractive. The advantage would arise by using solvent extraction to remove inhibitory organics and reduce the phenolics to a level amenable to biological treatment. Because phenol is a weak acid, the opportunity exists to selectively extract excess phenol from the wastewater. The remaining phenol could then be anaerobically converted to methane (a recoverable fuel). The opportunity also exists to recover the spent solvent by the distillation process.

While the role of GAC organic adsorption has been shown to improve the performance of anaerobic biological systems, the importance of biomass retention in the anaerobic process has also been stressed. A better understanding of the fundamentals of attached-growth processes and related techniques for promoting biomass retention may lead to improved anaerobic GAC treatment of phenolic wastewaters.

2.3 Microorganism-Support Material Association

Providing a support material to allow attachment and retention of the slow-growing anaerobic bacteria offers the opportunity to enhance the biological process performance. To evaluate this potential it is necessary to examine the fundamentals of microorganism-support material interaction.

2.3.1 Features of the Microbe-Medium-Support Material System

The association of microorganisms with solid surfaces results from the interaction of physical and chemical characteristics of:

i) the support material surface,

ii) the aqueous environment, and

iii) the microorganisms.

The actual mechanisms of microbial adhesion and attachment to a surface will be discussed in section 2.3.2.

2.3.1.1 Characteristics of a Biological Support Surface

a) Surface Free Energy

An important physicochemical property of a support material is the surface free energy (Fletcher and Marshall, 1982) because it comprises all the surface forces capable of interacting with forces associated with the adjacent phases (i.e. the liquid medium and bacteria surface). The free energy of the surface is the available energy resulting from the interaction of atoms and molecules on the surface with atoms and molecules which may approach the surface. The types of interactions that may arise are:

i) van der Waals dispersion forces (weak charge attraction forces resulting from the fluctuating distribution and temporal-spatial concentration of electrons in atoms and molecules),

ii) electrostatic interactions between charged groups,

iii) polar interactions between groups with permanent or induced dipoles, and

iv) chemical bonding (ionic, covalent, and hydrogen bonding).

The molecules within the bulk of the support material can interact with surrounding molecules, whereas those at the surface interact with ones beneath them in the bulk material and with molecules in the adjacent phases. The free energy at the surface will be greater than that of the interior because of the greater interaction opportunities for molecules at the surface with molecules (of different origin) approaching the surface.

Surface free energy is important to bacterial attachment because bacterial adsorption on the support material surface reduces the free energy of the entire system. A natural tendency of any system is to minimize the free energy within the system, and in doing so approach a state of thermodynamic equilibrium.

b) Surface Charge

The surface will tend to accumulate and concentrate particles and molecules from the liquid phase in a manner similar to that described above (Ellwood et al., 1982). Also, most solid surfaces immersed in water will assume a net negative charge (Marshall, 1979), and as a result attract cations to the surface. This will further alter the surface free energy and have an effect on the behavior of organisms approaching the surface.

c) Surface Area

Kolot (1981) lists surface area as an important criteria for the selection of biological supports. The larger the support surface area is the greater the opportunities for bacteria to attach and colonize the surface. Zobell and Anderson (1936) found that the number of bacteria present in stored sea water and rate of carbon mineralization was proportional to the surface area to volume ratio of the container used.

d) Surface Roughness

The surface roughness (e.g. projections, pits, and crevices) plays an important role for attached bacteria because (Fletcher and Marshall, 1982):

i) it increases the convective mass transport of bacteria, substrate, and nutrients near the surface,

ii) it increases the surface area for attachment, and

iii) it provides shelter or protection for bacteria from fluid shearing forces or abrasion.

Weise and Rheinheimer (1978) found that bacteria tend to colonize more in crevices than on ridges of marine sand sediments.

e) Pore Volume

The internal pore dimensions are found to play an important role in accumulating biomass on biological supports. Messing et al. (1979) found a relationship between the accumulation of viable biomass and the pore morphology of a stable inorganic biological support. Bacteria were presumed to colonize in the pores of the support that were large enough to accommodate them. The pores were assumed to protect attached bacteria from fluid shear forces that may exist in the bulk liquid.

2.3.1.2 Aqueous Environmental Conditions

The important characteristics of the aqueous environment relative to bacterial attachment are:

i) pH,
ii) electrolyte concentration, and
iii) hydrodynamic factors.

pH is thought to play an important role in that support materials which contain acidic surface functional groups will ionize to a degree dependent upon the solution pH. This will determine the surface charge and the extent to which ions of opposite charge (counter-ions) are attracted (Ellwood et al., 1982). Engineered biological wastewater treatment systems are generally designed to maintain pH near neutrality for optimum biological activity and, therefore, define the role pH will play in encouraging microbial attachment. The electrolyte concentration of the aqueous phase affects the surface charge of immersed support material by allowing the adsorption and accumulation of ions and molecules and imparts a surface charge (Fletcher et al. 1980).

Hydrodynamic factors (fluid motion and mixing) provide transport of ions, molecules, and bacteria to within close range of the support surface. Such features are also important in transporting substrate and nutrients to the surface for attached bacteria. Once bacteria have colonized the surface and developed a biofilm, liquid motion (in this case shearing effects) adversely affects the biofilm by shearing off bacteria in direct contact with the bulk liquid. Surface area or internal pore volume that is free from liquid shear should promote attached-biomass development providing there is access to the food and nutrient supply from the bulk liquid or migrating adsorbed organics.

2.3.1.3 Microorganism Characteristics

The surface charge of microbial cells can play an important role in attachment to surfaces. Most bacteria bear a net negative charge (Fletcher and Marshall, 1982). Since most support surfaces are negatively charged (Marshall, 1979), long range repulsion forces are created and become significant in the initial process as bacteria adsorb to the surface.

2.3.2 Adhesion and Attachment of Microorganisms to Solid Surfaces

The accumulation of nutrients at the surface, the fact that attached-bacteria expend less energy seeking out nutrients and food than motile bacteria, and the thermodynamic driving force to reduce the overall free energy of the system by adsorbing particles (including bacteria) to the surface appear to make it advantageous (or inevitable) that microbial adhesion and attachment to a surface will occur. The attachment of bacteria to solid surfaces will involve an interaction between the surface and the outer layer of the bacteria.

Once bacteria have been transported to the immediate vicinity of the surface, the physicochemical and biological attraction mechanisms to the surface are (Marshall, 1976):

i) chemotaxis (movement of microorganisms toward or away from a chemical stimulus),

ii) Brownian motion (random motion caused by molecular and particle collisions in water),

iii) electrostatic attraction (surface charge of the solid as "seen" by bacteria may have different ionic properties than anticipated due to counter-ion accumulation),

iv) electrical double layer effects (under high electrolyte concentration a net attraction between the bacteria and surface will exist - to be discussed later), and

v) cell-surface hydrophobicity (the outer surface of some bacteria is hydrophobic).

The three stages of microbial adhesion to a surface are (Marshall, 1976):

i) adsorption,

ii) firm attachment, and

iii) colonization (formation of biofilm).

2.3.2.1 Adsorption

As negatively-charged bacteria approach a negatively-charged surface (Figure 2.3), the interaction of the two surfaces is best examined by the DLVO theory of colloid stability (Verwey and Overbeek, 1948). The energy of interaction that arises between the two surfaces is the sum of the electrical double layer repulsion force and van der Waals dispersion (or attraction) force (see Figure 2.4a). The electrical double layer consists of a charged particle surface and an equivalent excess of counter-ions which accumulate in the water near the surface (Weber, 1972). The electrical double layer repulsion arises when two similar charged surfaces approach each other.

The counter-ion accumulation near both the solid and bacteria surfaces increases under conditions of high electrolyte concentration and, as a result, the electrical double layer repulsion force is compressed. This allows bacteria to approach closer to the surface. A secondary attraction minimum occurs (see Figure 2.4b) leading to speculation that attraction to and adsorption of bacteria occurs at this point (Marshall, 1980).

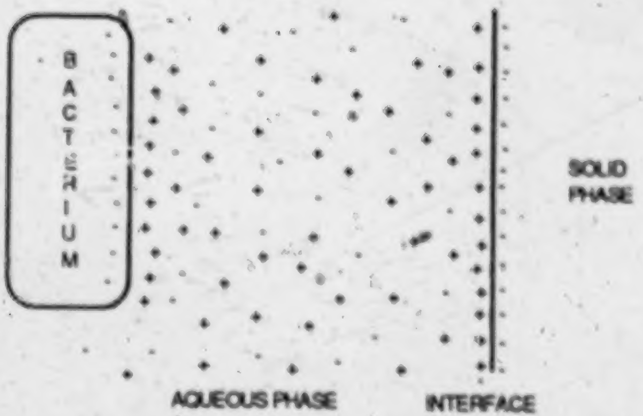
BACTERIUM
AQUEOUS PHASE
INTERFACE
SOLID PHASE

Figure 2.3 Charge Interaction Between Bacteria and a Solid Support

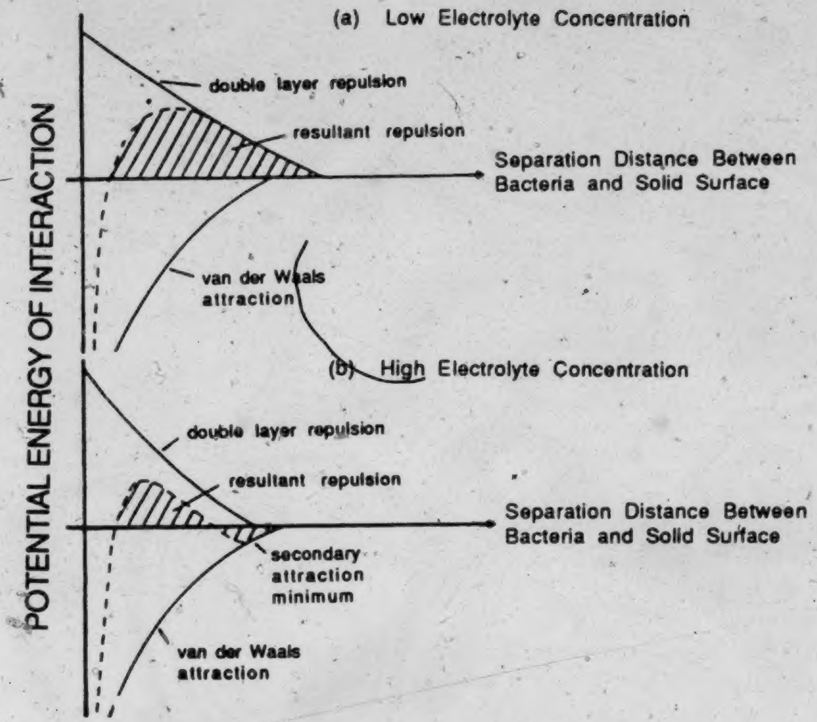
(a) Low Electrolyte Concentration
double layer repulsion
resultant repulsion
Separation Distance Between Bacteria and Solid Surface
van der Waals attraction
(b) High Electrolyte Concentration
double layer repulsion
resultant repulsion
Separation Distance Between Bacteria and Solid Surface
secondary attraction minimum
van der Waals attraction
POTENTIAL ENERGY OF INTERACTION

Figure 2.4 Potential Energy of Interaction Between Bacteria and Surface at Low and High Electrolyte Concentration (Adapted from Marshall, 1976)

Van Haecht et al. (1985) demonstrated that the adsorption of aluminum ions (Al^{3+}) on the surface of yeast cells decreases the negative electrostatic potential (electrical double layer repulsion force) near the cell surface. This allowed enhanced adhesion of the cells to a negatively charged glass surface. Provided the adsorbed microorganisms are not subjected to strong hydraulic shear forces near the surface, firm attachment can take place.

2.3.2.2. Firm Attachment

The firm attachment of bacteria to a surface is the process of anchoring the bacterium. Costerton et al. (1978) have shown that bacteria produce extracellular polysaccharide fibers which are used to firmly anchor the cell to the surface. The actual anchoring mechanism is still unknown. The polysaccharide fibers are mainly negatively charged and divalent cations (e.g. Ca^{2+} and Mg^{2+}) apparently act as linkages between the negatively charged surface and microorganism fibers (Bryers, 1987; and Stronach et al., 1986).

2.3.2.3 Colonization

Colonization or biofilm development occurs as attached organisms grow (proliferate) and produce a mass of polysaccharide fibers that surround an individual cell or colony of cells. This felt like "glycocalyx" (Costerson et al., 1978; 1981) acts as an ion exchange resin to attract and concentrate charged nutrients, and, also to attract secondary colonizers who anchor themselves to the glycocalyx (Costerton, 1984).

2.3.3 Studies of Biologically Active Surfaces

The advantages of using attached growth biological systems over free suspension systems are well established. Bryers (1987) summarized the advantages of attached growth systems. They:

i) facilitate biomass-liquid separation and biomass concentration,

ii) provide high reactor cell concentration at any residence time,

iii) increase overall system productivity and minimize the wash-out effect,

iv) protect against biomass loss due to system-hydraulic upsets,

v) provide the possibility to spatially locate different desired populations within a process,

vi) supply a necessary substratum for growth, and

vii) allow the possibility of using diffusional limitations in the situation of substrate-inhibition kinetics to enhance overall system productivity.

Doran and Bailey (1986) demonstrated that immobilized yeast cells had altered metabolism that allowed enhanced fermentation capabilities compared to suspended yeast cells. The immobilized cells consumed glucose twice as fast as the suspended cells, but their specific growth rate was reduced by 45%. The attached-bacteria require less expenditure of energy than suspended-bacteria in obtaining nutrients and food and, therefore, operate more efficiently.

Wilkie and Colleran (1984) and Wilkie et al. (1985) investigated the effect of four different support materials during the startup of anaerobic filters with pig slurry supernatant. Plastic rings, coal, mussel shells, and fired clay fragments were used in 21.3 L reactors. The authors used %COD removal as a measure of the biological activity in each reactor. The pig slurry had a COD of 29,730 mg/L (13,790 mg/L volatile organic acids). The support material surface areas and COD removals for each filter are presented in Table 2.14. The data presented for %COD removal at day 2 and 5 after startup show that materials with higher surface area did not exhibit the best process performance. The reactor containing fired clay fragments had the lowest total matrix surface area and was able to develop the greatest amount of bioactivity more rapidly than the other reactors. By day 70 all reactors were achieving similar COD removals. The advantage of the clay matrix in the early stage may arise because its surface carries a high negative charge and, therefore, attracts a significant cation concentration (Stumm and Morgan, 1981). As discussed earlier, attraction to and adsorption of bacteria to the surface can readily occur under these conditions (providing a high electrolyte concentration exists). Consequently, the reactor containing fired clay frgaments may have developed active attached-biomass faster than the other reactors due to improved initial adsorption and attachment of bacteria.

Robinson et al. (1984) observed the distribution and types of anaerobic microbes present in biofilm removed from digestors treating fresh swine manure. The digestors contained various types of plastic and wood support materials and had been in operation for

2

Table 2.14 Anaerobic Filter Characteristics and Performance During Pig Slurry Supernatant Digestion (After Wilkie et al., 1985; Wilkie and Colleran, 1986)

	Support Material			
	Clay	Coral	Mussel Shell	Plastic
Matrix specific surface area (m^2/m^3)	119.0	490.0	161.0	179.0
Total matrix surface area in reactor (m^2)	2.142	8.820	2.898	3.222
% COD removal (day 2)[1]	36	15	8	10
% COD removal (day 5)[1]	48	32	17	26
% COD removal (day 70)	73	70	69	73
Effluent VOA (mg/L)[2]	584	1,626	1,525	1,385

[1] % COD removal was estimated from data presented from authors.

[2] Effluent volatile organic acid average concentration from day 65-75.

a 12 month period. They observed that the biofilm growth on the support materials did not differ in microbial content or overall appearance and was 1 to 3 mm thick on all of the support materials. The 12 month period was sufficiently long enough for biomass to completely colonize the surface of all the support materials.

Kennedy and Droste (1985) investigated the use of different support materials in the startup of anaerobic downflow stationary fixed-film reactor treatment of bean blanching wastewater (9-11 g/L COD). They found that materials with roughened surfaces gave the best performance during startup. Support materials such as needle-punched polyester and fired clay fragments allowed quicker biofilm development than PVC or glass support material. The authors felt that bacteria, especially the methanogens, had difficulties adhering to the smooth PVC or glass surfaces. Increased material surface area in the reactors led to more rapid increases in organic loading rates and higher ultimate loadings. The reactors with higher loading rates achieved higher rates of methane gas production. This study demonstrated that surface area is important for biofilm development.

Work by Bryers and Characklis (1982) examined the process of biofilm formation. They found that adsorption of bacteria to surfaces was influenced by the initial suspended-biomass concentration. The suspended-biomass deposition rate (i.e. combined transport and adsorption rate) was directly proportional to the suspended-biomass concentration which suggested that deposition was controlled by particle transport to the surface rather than cellular adhesion. They also reported that the deposition rate was unaffected by a doubling of the Reynolds number (a numerical index used to characterize the type of liquid flow). However, once the biofilm developed, biofilm detachment increased with increasing Reynolds number.

Verrier et .al. (1987) studied the initial adsorption of four methanogenic bacteria on polymeric surfaces of different hydrophobicities. Table 2.15 summarizes their findings. The high hydrophilic nature of the extracellular polymers excreted by *Methanosarcina mazei* was given as the reason for its non-adherence. The electrical surface charge of the other methane bacteria accounted for their behavior. *Methanospirillum hungatei* was hydrophilic in nature and preferred more hydrophilic surfaces. *Methanothrix soehngenii* (hydrophobic) preferred more hydrophobic surfaces, while *Methanobrevibacter arboriphilicus* preferred all supports tested. It should be pointed out that all of the methanogens could be present in any biofilm that forms on these surfaces from a mixed culture (by attaching as a secondary colonizers to the glycocalyx of attached-biomass).

Kuroda et al. (1988) examined the adhesion of methane- and acid-forming bacteria to solid support materials. The materials tested included glass, stainless steel, carbon, ceramic, resin, zeolite, diatomaceous earth, and plasticized alumina fibrous material. They characterized the support materials by the internal pore diameter size. The best adhesion and biofilm characteristics were observed on solid supports made of carbon material containing pore diameters of 150-300 µm. They concluded that biofilm development was dependent upon the growth of bacteria, glycocalyx

Table 2.15 Adhesion Preference of Selected Methanogenic Bacteria[1] (After Verrier et al., 1987)

Bacteria	Adhesion Preference
Methanosarcina mazei MC3	None of the supports
Methanothrix soehngenii	Hydrophobic surfaces
Methanospirillum hungatei JF1	Hydrophilic surfaces
Methanobrevibacter arboriphilicus AZ	All of the supports

[1] Support materials tested (in order of decreasing hydrophobicity):

polytetrafluorethylene - more hydrophobic surface
polypropylene -
polyethylene -
polyvinyl chloride -
polyacetyl -
polyamide 6 - more hydrophilic surface

production, and the fluid flow regime rather than on the support surface characteristics. However, as shown earlier (section 2.3.1.1), the surface chemical characteristics are important during the initial adsorption step.

Selle Sardi et al. (1986) investigated the adhesion and growth of anaerobic biofilms on ion exchange resins. They found that strong cation exchange resins were preferred for anaerobic biofilm development due to their favorable property of divalent cation binding and adsorption of volatile organic acids. Divalent cations are thought to aid in the adsorption and firm attachment of bacteria to firm supports. The adsorption of volatile organic acids to the resin surface may have created a favorable substrate environment at the surface for bacteria (especially methanogens).

Van Haecht et al. (1985) investigated the adsorption of aluminum ions (Al^{3+}) to yeast cells and the subsequent interaction with a glass surface. The cells were initially contacted with an Al^{3+} solution, then a glass microscope slide was immersed in the mixture. The slide was then washed with demineralized water. The authors found that the Al^{3+} treated cells readily adsorbed to the glass slide. The authors suggested the presence of Al^{3+} ions adsorbed to the cell surface decreased the negative surface charge and allowed the cell to adsorb to the negatively charged glass more easily than in the absence of the Al^{3+} ions.

In recent years, studies have been conducted with activated carbon as a biological support and an adsorbent for the anaerobic treatment of phenolic wastewaters. Activated carbon has an adsorptive capacity for phenols and, as such, may create a favorable substrate environment for phenol-degrading bacteria. Also, activated carbon may aid in the removal of inhibitory organics that are typical of many phenolic wastewaters.

Activated carbon is made by a pyrolytic process that results in a microcrystalline structure composed of parallel graphite planes stacked in an unordered fashion (McGuire and Suffet, 1978). During the process, oxygen is chemisorbed to the surface to give it much of its associated functionality. Adsorption is a surface phenomenon and the adsorption or organics (or inorganics) at the surface of activated carbon involves the minimization of surface free energy (Weber, 1972). It is, therefore, not suprising that activated carbon would favor the adsorption of bacteria to its surface (see section 2.3.1.1).

Laboratory reactor studies of anaerobic phenolic treatment have reported that GAC is superior in-situ material when compared to non-activated carbon material (Gardner et al., 1988), plastic media (Suidan et al., 1983b), and anthracite coal (Khan et al., 1982). In all situations the wastewaters tested contained a background matrix of unknown potential organic inhibitors. The superior performance of the activated carbon reactors in these studies was probably more a result of the GAC adsorption and removal of organic inhibitors. Little or no direct information regarding the usefulness of GAC as a biological support was offered in these studies.

The mechanisms by which GAC provides superior anaerobic treatment of phenolic wastewaters are speculated to be (Suidan et al., 1981a; Khan et al., 1982; Suidan et al., 1983a and Kim et al., 1986):

i) the adsorption properties of activated carbon allow it to buffer high concentrations of substrate which may exceed the tolerance range of anaerobic bacteria,

ii) the large surface area afforded by activated carbon promotes biomass growth. This may be caused by the presence of crevices, pores, and other surface irregularities that provide microbial attachment sites and offer protection from hydraulic shearing forces,

iii) the adsorptive properties of activated carbon increase the concentration of substrate at the liquid-solid interface and promote biological growth, and

iv) activated carbon has an adsorptive capacity for the extended retention of inhibitory or less readily biodegradable compounds.

A favorable characteristic of activated carbon-biomass system is the bioregeneration capacity which can occur during the interaction of microorganisms with organic substrates (Kim et al., 1986). Activated carbon buffers microorganisms from organic substrate shock loads through rapid initial adsorption into pores and slow subsequent release by desorption of the organic substrates. Desorption would occur under conditions where the bulk liquid concentration of the organic substrate decreased. The desorption sites would then be available for further shock loading conditions. The desorption accompanied by biodegradation of the desorbed substrate is referred to as bioregeneration. Pores large enough to accommodate bacteria would have continuous exposure to substrate while being free from hydraulic shearing forces under these conditions.

2.3.4 Discussion of Biological Support Material

The ideal features of a support material should encourage both the initial adsorption of bacteria to the surface and allow the unhindered development of biofilm. Engineered biological processes employing attached-growth organisms are intended to maintain well developed biofilms. This is especially important for the anaerobic process because of its low biomass production rate. Support material features that encourage biofilm development and maintenance appear to offer the best opportunities for enhancing productivity of anaerobic processes.

The features of attached-growth systems which encourage the adsorption of anaerobic bacteria to a support material should focus on the physical/chemical characteristics of the support material and liquid environment and not on the microorganism characteristics. This is because a diverse microbial population may be required to allow the effective degradation of phenolics (see Section 2.1.2) and not all microorganisms will have strong affinity for surfaces. The selective culturing of the minimum number of species required to achieve phenolic degradation is likely an impractical approach for full scale anaerobic systems. However, ensuring optimum support material and liquid medium features can be achieved.

Support materials that possess a high free surface energy in an aqueous environment will encourage adsorption of bacteria to the surface. Also, a high surface area should be available to allow greater opportunities for adsorption. The electrolyte (divalent cation) concentration in the medium should be sufficient to promote the adsorption, attachment, and colonization of the bacteria. A high seed concentration of suspended biomass should be present to promote adsorption to the surface of support materials (Bryers and Characklis, 1982).

Allowing unhindered biofilm development is important in anaerobic processes in order to retain the slow-growing microorganisms and provide maximum bioactivity. Preferred support materials are ones that would offer the developing biofilm easy access to food and nutrients while protecting the biofilm from fluid shearing forces. Materials with highly irregular surfaces (i.e. pits, ridges, and crevices) and well-developed internal pore structure (large enough to accommodate bacteria) would be ideal. It is important that bacteria which colonize these areas have ready access to the food and nutrient supply from the bulk liquid.

Activated carbon may provide many of the features discussed above, and more for anaerobic attached growth treatment of phenolic industrial wastewater. Specifically, activated carbon has an adsorptive capacity for the extended retention of inhibitory or less readily biodegradable compounds. The adsorption properties also allow activated carbon to buffer high concentrations of phenol which may exceed the tolerance range of bacteria. The adsorptive properties would tend to moderate biological inhibition under these conditions.

The available free surface energy of activated carbon is ideally suited for adsorption of bacteria, and the surface area may offer good potential for biofilm development. Most important is biofilm persistence. The internal pore structure of activated carbon may provide an excellent environment for this. Pores large enough to accommodate biofilm would have continuous exposure to phenolics (substrate) traversing both directions in the pores (inwards during adsorption and outwards during desorption). Pores would also offer the biofilm protection from the bulk liquid shearing forces.

The bioregeneration property of an activated carbon-biomass system would protect microorganisms from shock substrate loads through rapid initial adsorption into pores and slow subsequent release by desorption of phenolics (substrate). This mechanism may promote good conditions for initial system startup (biofilm development) and offer the best protection against biological upset.

2.4 Research Objectives

This research will examine the combined treatment requirements for an industrial phenolic (coal conversion) wastewater and will focus on the mechanisms by which activated carbon acts as a biologicial support during anaerobic degradation of phenol. Specific objectives are to:

1) Investigate the potential of solvent extraction of a coal liquefaction wastewater for the selective control of biodegradable phenolics and selective removal of non-phenolic organics which may be inhibitory.
2) Examine the resulting anaerobic biodegradation potential of phenol and m/p-cresol in the solvent extracted wastewater and evaluate the options for maintaining effective treatment activity on a continuous basis.
3) Characterize the surface chemical and physical features of selected biological support materials (activated carbon and synthetic ion exchange resin) for the purpose of optimizing anaerobic biofilm development and performance during the degradation of phenol.
4) Determine the role which the support material plays during anaerobic phenol degradation.

3.0 EXPERIMENTAL TECHNIQUES

3.1 Anaerobic Culture Methods

3.1.1 Serum Bottle Procedures

All anaerobic biological assays were performed using the serum bottle modification of the Hungate method (Miller and Wolin, 1974). Serum bottles of 58 or 119 mL volume (Wheaton Scientific) were submerged in warm water, inverted, and filled with 30% CO_2 in N_2. The gas mixture was free of oxygen by passing it through a heated glass column filled with copper filings (Hungate, 1969). The serum bottles were then removed from the water, positioned upright, and allowed to sit for 3 to 5 min while continuously flushed with the gas mixture.

The addition of growth medium, inoculum, and feed solutions to the serum bottles were performed anaerobically with flushed volumetric pipettes or plastic syringes fitted with disposable needles (Becton-Dickenson; B-D). The pipette or syringe was filled with the gas mixture several times before use, thus minimizing the potential for oxygen contamination. Inoculum and feed solution addition by the syringe and needle method could be performed either before, or after the serum bottles were sealed. The serum bottles were closed with a butyl rubber stopper and sealed with a crimped metal seal.

3.1.2 Biological Growth Medium

The growth medium was made from stock solutions of macronutrients (cation salts and phosphate), micro-nutrients (trace metals), B vitamins, a redox indicator (resazurin), and an aqueous reducing agent (sulfide). Table 3.1 gives the concentrations of each solution.

The stock solutions were added to a flask containing 1,000 mL tap water according to Table 3.2. The flask was heated to a boil on a hot plate and cooled to room temperature with approximately 900 mL solution remaining. The flask headspace was continuously flushed with O_2-free 30% CO_2 in N_2. 100 mL of a $NaHCO_3$ solution (40 g/L) was added to the flask along with 5 mL of the sulfide solution (Table 3.1). The bicarbonate solution was prepared in freshly boiled water just prior to use. The final pH of the growth medium solution was checked and adjusted (if necessary) to 6.9-7.2 with NaOH or HCl. The solution was sufficiently reduced to allow transfer and storage (for extended periods of time at 20°C) in 119 mL serum bottles.

3.2 Analytical Methods

3.2.1 Acidity

Acidity titrations were performed potentiometrically with a Fisher Accumet pH meter (Model 805 MP) according to standard methods (APHA 1985, section 402). A 25 mL sample was pipetted from a freshly drawn sample and titrated with 0.2 N NaOH.

Table 3.1 Stock Solutions Used in Anaerobic Growth Medium

Solution	Chemical	Concentration in Distilled H_2O (g/L)
Mineral I(1)	$NaCl$	50
	$CaCl_2 \cdot 2H_2O$	10
	NH_4Cl	50
	$MgCl_2 \cdot 6H_2O$	10
Mineral II	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	10
	H_3BO_3	0.3
	$ZnSO_4 \cdot 7H_2O$	0.1
	$FeCl_2 \cdot 4H_2O$	1.5
	$CoCl_2 \cdot 6H_2O$	10
	$MnCl_2 \cdot 4H_2O$	0.03
	$NiCl_2 \cdot 6H_2O$	0.03
	$AlK(SO_4)_2 \cdot 12H_2O$	0.1
Vitamin B	Nicotinic acid	0.1
	Cyanocobalamine	0.1
	Thiamine	0.05
	p-Aminobenzoic acid	0.05
	Pyridoxine	0.25
	Pantothenic acid	0.025
Phosphate	KH_2PO_4	50
Resazurin		0.1
Sulfide	$Na_2S \cdot 9H_2O$	25

(1) Dissolved in 0.01 N HCl.

Table 3.2 Anaerobic Growth Medium Composition

Solution[1]	Volume per Litre Tap Water (mL)
Mineral I	20
Mineral II	2
Vitamin B	2
Phosphate	20
Resazurin	1

[1] See Table 3.1.

3.2.2 Volatile Suspended Solids

Volatile suspended solids (VSS) were determined by standard methods (APHA 1985, section 209). A 10 mL sludge sample was placed in an aluminum evaporating dish and oven dried at 105°C until dryness. The aluminum dish had previously been fired at 550°C for 30 min, cooled to room temperature, and tared. After the oven dried sample had been cooled in a dessicator and weighed, it was fired at 550°C for 30 min, cooled to room temperature, and weighed again. All determinations were made in triplicate and average values were recorded.

3.2.3 Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined using the dichromate reflux method described by standard methods (APHA 1985, section 508a). All samples were analyzed in duplicate. Most samples were diluted with distilled water to bring the COD value within the test range.

3.2.4 Volumetric Methane Measurements

The absolute quantity of methane produced in the serum bottle headspace was obtained in the following manner. Total gas volumes at 37°C were measured by displacement of a glass barrel in a 30 mL lubricated gas tight syringe (B-D) fitted with a 22G x 38 mm needle. The headspace gas was periodically flushed with O_2-free 30% CO_2 in N_2 to allow measurement of methane over a long period of time.

Methane concentrations in the headspace gas were determined by gas chromatography (GC). A Hewlett-Packard Model 5730A GC equipped with a flame ionization detector (FID) was used. A 0.1 mL gas aliquot was injected onto a 0.65 m x 2 mm ID glass column containing 1% SP1000 60/80 Carbopack B (Supelco). The resulting peak areas were compared to those from injections of standards of known concentration (Kindzierski, 1984). Nitrogen was used as the carrier gas at 30 mL/min. Hydrogen and air were used as the flame detector gases at 30 and 240 mL/min respectively. The injector, oven, and detector temperatures were 25, 25, and 250°C respectively.

The methane concentrations were corrected for the presence of water vapor at 37°C (Kindzierski, 1984). Methane sampling and removal was performed with 1 mL plastic syringes equipped with a 26G x 15.9 mm needle (B-D).

3.2.5 **Phenolics - HPLC Analysis**

High pressure liquid chromatography (HPLC) analysis was performed by Joyce Hudson, Department of Civil Engineering, University of Alberta, Edmonton, Alberta. Phenol and cresol compounds were analyzed by HPLC using a Hamilton PRP-1 4.1 mm x 150 mm reverse phase column. This method did not resolve m-cresol from p-cresol. A Waters Associates chromatography pump (M-600A) was used for flow control. Compound detection was achieved with a Model 1040A Diode Array Detector (Hewlett-Packard) operating in the ultraviolet wavelength range (210 nm). The mobile phase was 1:1 acetonitrile:distilled water which exhibited negligible absorptive interference at the chosen detection wavelength. The mobile phase was degassed prior to use by vacuum filtration through a 0.45 µm nylon membrane filter.

Serum bottles containing phenolics were sampled after inverting for 20 min to settle the solids. Then a 1 mL plastic syringe equipped with a 22G x 38 mm needle was inserted through the serum stopper, above the settled solids, and 0.3 mL of supernatant was withdrawn. The sample was placed in a polypropylene microfuge tube (Beckman) and centrifuged (10,000 RPM) for 45 min to further remove solids. The sample was then drawn into another 1 mL plastic syringe to which a 0.45 µm disposable filter assembly (ACRO LC3S, Gelman Sciences) was affixed. The syringe filter assembly was used to inject the sample onto a sample loop for introduction to the HPLC.

3.2.6 **Phenolics - GC Analysis**

Phenol and the cresols were also analyzed by GC using the technique of direct aqueous injection on 1% SP1000 60/80 Carbopack B (Supelco). The 0.65 m x 2 mm ID glass column was housed in Hewlett-Packard Model 5730A GC equipped with a FID. Nitrogen was used as the carrier gas at 30 mL/min. Hydrogen and air were used as the flame detector gases at 30 and 240 mL/min respectively. The injector, oven, and detector temperatures were 250, 210, and 250°C respectively. This GC method did not resolve the meta and para isomers of cresol.

Sampling was accomplished by inserting a 10 µL (Hamilton) syringe through the rubber stopper of an inverted serum bottle and withdrawing 3 µL of supernatant. From this aliquot 2 µL was directly injected onto the GC column. The resulting peak areas were compared against those from injections of phenol and cresol standards of known concentration.

3.2.7 **Diisopropyl Ether - GC analysis**

Diisopropyl ether (DIPE) was analyzed by GC using the direct aqueous injection technique and conditions described in section 3.2.6. Likewise, sampling was accomplished in the same manner.

3.2.8 **Gas Chromatography/Mass Spectroscopy**

Gas chromatography/mass spectroscopy (GC/MS) was used to tentatively identify compounds that were solvent extracted from the H-coal liquefaction wastewater. GC/MS work was performed by Susan Daignault, Department of Civil Engineering, University of Alberta, Edmonton, Alberta.

3.2.8.1 **Sample Workup**

The H-coal wastewater was adjusted to pH 9 and batch extracted with DIPE by contacting the wastewater with DIPE three times at a measured wastewater:solvent volume ratio of 10:1 (the exact extraction procedure is explained in section 4.1.6). The DIPE extracts were combined and aliquots were taken for further study. A 2 mL aliquot of the combined DIPE extract was back-extracted with 2 - 20 mL volumes of water adjusted to pH 12 with NaOH. The 2 mL fraction was then passed through a chromatography column containing 5 g of Florisil. The Florisil was eluted with acetonitrile (CH_3CN). The acetonitrile fraction was later analyzed by GC/MS. Fluorene (known concentration) was added to the acetonitrile fraction prior to GC/MS analysis to act as an internal standard.

3.2.8.2 **Operating Conditions**

A Varian 6000 gas chromatograph containing a 30 m x 0.25 mm DB-1 capillary column (J.W. Scientific) was coupled to a VG Analytical 7070E mass spectrometer equipped with a VG System 11/250 data processing unit. The mass spectrometer was operated in the electron ionization (EI) mode using a 70 eV source. The GC injection port temperature was 225°C and helium was used as the carrier at 1.5 mL/min. The oven temperature programming was adjusted as required to obtain the best component resolution and identification possible.

A computer library search was used to match mass spectra of interest with those stored in the data library system to tentatively identify the corresponding compounds. The original H-coal concentration was estimated for some of the major compounds identified. Instrument response of a compound was compared to the instrument response of a known concentation of internal standard (fluorene) to estimate the compound concentration in the acetonitrile fraction. The estimated H-coal concentration of the compound was then calculated assuming uniform extraction efficiency of the compound throughout sample workup and uniform instrument response for fluorene and all of the compounds.

3.3 Biomass - Characterization Methods

Several wet chemistry analytical methods were used to provide an estimate of the biological mass in serum bottle cultures.

3.3.1 Organic Nitrogen

Organic nitrogen content (intended as a measure of biological nitrogen) in anaerobic cultures was analyzed colorimetrically after a Kjeldahl digestion technique (APHA 1985, section 420). Analyses were performed by John Konwichi, Department of Soil Science, University of Alberta, Edmonton, Alberta.

The samples were block digested with sulfuric acid, potassium sulfate, and mercuric sulfate (a catalyst) to convert organic nitrogen to ammonium sulfate. Alkaline phenol and hypochlorite were then reacted with the ammonia (ammonium sulfate) to form an indophenol blue color that was proportional to the ammonia concentration. A Technicon Autoanalyzer (Technicon Instruments Corp.) was used to measure the sample color intensity at a wavelength of 630 nm.

Free ammonia and ammonium nitrogen was analyzed prior to the Kjeldahl digestion. The difference of Kjeldahl-nitrogen and ammonia-nitrogen represented organic nitrogen

3.3.2 Carbohydrate

Carbohydrate was analyzed by a modification of the colorimetric procedure described by Dubois et al. (1956). Carbohydrates react with phenol and concentrated sulfuric acid to form an orange color proportional to the concentration.

A sample aliquot and make-up distilled water (totalling 10 mL) were added to a 100 mL round bottom boiling flask along with 10 mL of a 6% phenol solution. Then 50 mL of concentrated sulfuric acid was rapidly added by directing the stream of the acid onto the surface of the liquid contents in the flask. The flask was swirled for 2 min after which 3 - 5 mL samples of the hot solution were transferred to disposable glass cuvettes. The cuvettes were placed in the dark for 45 min to allow the solution to cool to room temperature. Standards, derived from dilutions of a stock glucose solution (freshly prepared at 1,000 mg/L), and a distilled water blank were also carried through the acid digestion procedure. A Bausch and Lomb Spectronic 21D spectrophotometer was used to measure color intensity of the samples and standards at a wavelength of 485 nm.

3.3.3 **Protein**

Protein was analyzed using a dye-binding technique based on the differential color change in response to various concentrations of protein (Bradford, 1976). Samples were extracted with sodium dodecyl sulfate (SDS) to free the cell protein and assayed using the dye-binding technique.

A sample was extracted with 1% v/v SDS for 15 min. The sample-SDS solution was added to a test tube and placed in a sonicator (Branisonic Ultrasonic Cleaner, Model 2200). The test tube was then transferred to a table top centrifuge (International Clinical Centrifuge, Model CL) and spun at 3,500 RPM for 10 min to settle particulates and cell debris. The sample was removed from the test tube and diluted 10-fold to give a final SDS concentration of 0.1%.

An aliquot of the diluted solution (0.2 mL) was added to a disposable glass cuvette along with 5 mL of a dye-binding reagent (Bio-Rad, 1986). The cuvette was capped, inverted several times to mix, and placed in the dark for 15 min. Standards, derived from dilution of bovine plasma gamma gobulin (Bio-Rad, 1986) and a distilled water blank, containing 0.1% SDS were reacted with the dye-binding reagent in a similar fashion. All samples and standards were analyzed in triplicate. A Bausch and Lomb Spectronic 21D spectrophotometer was used to measure color intensity of the samples and standards at a wavelength of 595 nm.

3.4 Solid Support Material - Characterization Methods

Investigations of the support material surface characteristics were undertaken to identify features that may be important in determining the preference of microorganisms for a support material. A summary of characterization techniques used to study three support materials (activated carbon and two synthetic ion exchange resins) is provided in Table 3.3. Most of the techniques listed in Table 3.3, with the exception of elemental analysis and neutron activation analysis, have been employed previously to study the surface chemistry of carbon materials (including activated carbon; Kinoshita, 1988) and other adsorbent materials (Kane, 1986).

Table 3.3 Support Material Characterization Methods

Support Material Type	Surface Morphology (Physical)	Surface Chemistry (Chemical)
Activated carbon	MP NAI	DT(FTIR)S EA NAA PA(FTIR)S DR(FTIR)S XPS
Synthetic ion exchange resins	MP	*

DR(FTIR)S	-	Diffuse reflectance (Fourier transform infrared) spectroscopy.
DT(FTIR)S	-	Direct transmission (Fourier transform infrared) spectroscopy.
EA	-	Elemental analysis.
MP	-	Mercury porosimetry.
NAA	-	Neutron activation analysis.
NAI	-	Nitrogen Adsorption Isotherm.
PA(FTIR)S	-	Photoacoustic (Fourier transform infrared) spectroscopy.
XPS	-	X-ray photoelectron spectroscopy.
*	-	Information supplied by manufacturer.

3.4.1 Elemental Analysis

The elemental analysis was performed in a Perkin-Elmer Elemental Analyzer (Model 240) according to the technique of Cottrell and Cottrell (1977) to measure the elemental oxygen composition (% by mass) of activated carbon. The analysis was performed by Darlene Marlow, Department of Chemistry, University of Alberta, Edmonton, Alberta.

The analysis is a high temperature process where the sample is heated to 800°C and elemental oxygen is driven off as carbon monoxide gas, with subsequent oxidation to carbon dioxide. A thermal conductivity meter was used for carbon dioxide detection. The oxygen content was determined from the percent by mass content of carbon dioxide.

3.4.2 Mercury Porosimetry

Mercury porosimetry was used to determine the internal pore volume of the support materials. A Micromeritics mercury porosimeter ("Pore Sizer 9300") was used to find the cumulative pore volume associated with the pore size distribution of activated carbon and synthetic ion exchange resins (Micromeritics, 1979). Mercury porosimetry measurements were performed by Randy Mikula, Department of Energy, Mines, and Resources, Devon, Alberta.

The analysis is based on the principle that the pressure required to force mercury (Hg) into the pores of the support materials is inversely proportional to the size of the pores. Precise measurements of the pressure required to force Hg into the pores and the corresponding volume of Hg forced into the pores allow determination of the volume of pores of a given size and of the accumulated internal volume.

3.4.3 Neutron Activation Analysis

Neutron activation analysis (NAA) was used to determine the oxygen concentration (% by mass) of the activated carbon sample. NAA analysis was performed by Dennis Eg, Slowpoke Facility, University of Alberta, Edmonton, Alberta.

The following irradiation scheme was used. A 0.7 g sample of the activated carbon was packed in a 1.5 mL polyethylene vial and bombarded with a thermal neutron (n) flux of $1.0 \cdot 10^{11} \frac{n}{cm^{-2} \cdot s^{-1}}$. The irradiation ($t_{irr}$), decay ($t_{decay}$), and counting ($t_{count}$) time sequence was 10, 10, and 30 s respectively. The counting geometry (distance between the sample and detector) was 30 mm. Detection was accomplished with a high resolution gamma (¥) spectrometer.

The radionuclide ^{18}O (natural abundance 0.205%) was used for the determination according to:

$$^{18}_{8}O + ^{1}_{0}n \text{ ===> } ^{19}_{8}O + \text{¥}.$$

The oxygen concentration was expected to be determined by comparing the net counts from the 197 keV ¥ particles emitted by ^{19}O (half life of 26.9 seconds) of the activated carbon sample with the oxygen standard (K_2CO_3).

3.4.4 Nitrogen Adsorption Isotherm

The internal pore surface area of activated carbon was determined by the Brunauer, Emmett, and Teller (BET) method (Brunauer, 1938). This method utilizes N_2 gas adsorption for the evaluation of monolayer capacity (i.e. the volume of N_2 gas in cm^3 at standard temperature and pressure that would be required to cover the adsorbent with a monolayer; Faust and Aly, 1983). The nitrogen adsorption isotherm with activated carbon was performed by Jan Adamic, Department of Chemical Engineering, University of Alberta, Edmonton. Alberta.

The monolayer capacity was determined by performing an adsorption isotherm with N_2 gas on the activated carbon at liquid nitrogen temperature (195.8°C). An Omnisorb (Model 360) gas adsorption instrument (Omnicron Technology Corp.) was used. The activated carbon sample was heated at 300°C for 3 hours prior to analysis to remove moisture.

3.4.5 Direct Transmission (FTIR) Spectroscopy

Direct transmission Fourier Transform infrared spectroscopy (FTIR) was used for investigation of the chemical characteristics present on the surface of activated carbon. Absorption of mid-spectral infrared (IR) radiation (wavenumbers of 400 to 4,000 cm^{-1}) by atomic bonds on a sample surface can provide information of the type of chemical bond.

All spectra were recorded on a Nicolet 7199 FTIR instrument. The spectra were recorded with 32 scans collected at a resloution of 2 cm^{-1}. A blank KBr (Analytical Accessories Ltd., England) disc was measured with 32 scans and used as a reference. Spectra were recorded by Jim Hoyle, Department of Chemistry, University of Alberta, Edmonton, Alberta.

A modification of the KBr disc technique (Cook et al., 1954) was employed. Sample discs were prepared in the following manner:

1. Oven-dried (103°C) activated carbon of 75-150 µm average diameter was hand crushed with an agget mortar and pestle for 5 min.
2. Crystal IR grade KBr was hand crushed with an agget mortar and pestle and oven dried at 130°C for 1 h.
3. 5 g of KBr and 5.0 mg of activated carbon (both weighed to the nearest 0.1 mg) were transferred to the agget mortar and hand crushed (and mixed) for 5 min.
4. The mixture was oven dried overnight at 103°C and cooled in a dessicator prior to disc pressing.
5. 200 mg of the mixture (weighed to the nearest 0.1 mg) was transferred to a 10 mm diameter circular die and pressed at 2,068 KPa (30,000 psi) for 2 min.
6. The KBr discs were stored in a dessicator at room temperature prior to FTIR analysis.

3.4.6 **Photoacoustic (FTIR) Spectroscopy**

Photoacoustic (FTIR) spectroscopy (PAS) was used for investigation of the chemical characteristics present on the surface of activated carbon. In PAS, modulated radiation that is absorbed by a sample is converted into sound, which is then detected by a microphone (Kinoshita, 1988). The scattered light does not interfere with detection because only the absorbed light is converted into heat, which is then converted into acoustic waves. Absorption of mid-spectral infrared (IR) radiation (wavenumbers of 400 to 4,000 cm^{-1}) by atomic bonds on a sample surface can provide information of the type of chemical bond.

Activated carbon particles (75-150 µm) were analyzed with a Bruker FT (Fourier Transform) Spectrometer. Acoustic detection was achieved with a Princeton Applied Research Corp. Photoacoustic Accessory (Model 6003). The spectra was recorded with 1000 scans collected at a resolution of 4 cm^{-1}. Spectra was recorded by Curt Michealian, Department of Energy, Mines, and Resources, Devon Alberta.

3.4.7 Diffuse Reflectance (FTIR) Spectroscopy

Diffuse reflectance (FTIR) spectroscopy was used for investigation of the chemical characteristics present on the surface of activated carbon. Diffuse reflectance spectroscopy measures the radiation reflected from a sample surface with the aid of a modified transmission spectrophotometer (Frei and MacNeil, 1973). The data are reported as percent reflectance (%R) based on transmitted radiation and correspond to :

$$R = \frac{I}{I_o}, \text{ where:} \qquad \text{(Eqn. 3.1)}$$

I_o is the intensity of the incident radiation, and

I is the intensity of radiation reflected from the sample. Absorption of mid-spectral infrared (IR) radiation (wavenumbers of

400 to 4,000 cm^{-1}) by atomic bonds on a sample surface can provide information of the type of chemical bond. The relationship between absorbed (A) and transmitted (T) radiation is given by the Beer-Lambert law (Harris and Kratochvil, 1981):

$$A = \log\left[\frac{100}{\%T}\right]. \qquad \text{(Eqn. 3.2)}$$

Activated carbon particles (<75 µm) were analyzed with a Bruker FT (Fourier Transform) Spectrometer. Infrared detection was achieved with a Harrick Diffuse Reflectance Accessory. The spectra was recorded with 1000 scans collected at a resolution of 4 cm^{-1}. Spectra was recorded by Curt Michealian, Department of Energy, Mines, and Resources, Devon Alberta.

3.4.8 X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) is a form of ESCA (electron spectroscopy for chemical analysis) where x-ray irradiation of a solid surface can provide chemical information of the surface. X-ray photons are directed onto the sample surface and cause the ejection of an atomic core electron for every photon absorbed (Riggs and Parker, 1975). Electrons from all the orbitals of atoms with a binding energy less than the incident x-ray photon energy become excited. Because energy is conserved, the kinetic energy of an ejected electron plus the energy required to remove it from its orbital is equal to the incident x-ray photon energy. That is to say, the binding of an electron in an atomic orbital can be determined if its kinetic energy during ejection is measured and the incident x-ray photon energy are known (Seah and Briggs, 1983). Since the atomic orbital structure of each element is distinct, measurement of the binding energy of one or more orbital electrons allows the identification of that element present at the sample surface. The intensity of the measured signal (binding energies) observed is a function of the amount (or concentration) of element present on the surface.

XPS analyses were performed by Ross Davidson, Surface Science Laboratory, University of Western Ontario, London Ontario. The activated carbon sample was analyzed in a Surface Science Laboratories Model SSX-100 XPS using a monochromatized aluminum K alpha x-ray source. Widescan survey spectra were collected employing a 1000 µm x-ray spot size and high resolution narrow scan surveys on oxygen (1s) and carbon (1s) peaks were carried out with a 150 µm x-ray spot size. The analytical chamber vacuum was approximately $5 \cdot 10^{-9}$ torr. The sample was placed in a special holder and analyzed without electron flood gun compensation.

3.5 Support Materials For Biomass - Characterization Methods

Short term microbial adsorption studies (<4 h) and long term anaerobic biofilm growth characteristics (>90 days) were observed with the different support materials.

3.5.1 Radiolabelled Cell Adsorption Studies

Suspensions of ^{14}C radiolabelled *Pseudomonas aeruginosa* were contacted with different concentrations of the support materials for 2- and 4-h periods. The adsorption studies were performed by Neil Fairbairn, Department of Microbiology, University of Alberta, Edmonton, Alberta. The adsorption behavior of the cells were analyzed and interpreted according to the Freundlich isotherm equation.

3.5.1.1 Preparation of ^{14}C-Labelled *Pseudomonas aeruginosa*

1. "*P. aeruginosa* Minimal Medium" preparation:

i) $NH_4H_2PO_4$ (1.5 g), K_2HPO_4 (2.0 g), and $FeSO_4 \cdot 7H_2O$ (5 mg) were added to a 500 mL flask along with 300 mL distilled water, $MgSO_4 \cdot 7H_2O$ (0.5 g) was added to a 250 mL flask along with 100 mL distilled water, and glucose (2.5 g) was added to a 250 mL flask along with 100 mL distilled water.

ii) The above solutions were autoclaved (121°C for 20 min).

iii) After cooling to room temperature, the three solutions were aseptically transferred and mixed in a sterile 1000 mL flask.

iv) Two - 100 mL portions of the mixture were aseptically dispensed into 250 mL autoclaved flasks.

v) One of the flasks was inoculated with a loopful of *P. aeruginosa* from a petri dish culture grown on plate count agar (Difco) and incubated overnight on a shaker table (200 RPM) at room temperature.

vi) On the next day, 10 mL of the incubated culture was transferred to the second 100 mL aliquot along with 50 µL of 200 µCi/mL ^{14}C-glucose (100 µL of the this culture contained radioactivity ≈2.02•10^{4} dpm - disintegrations per minute).

vii) The radioactive culture was incubated overnight at room temperature on a shaker table (200 RPM) in a fume hood ($^{14}CO_2$ is produced).

2. Harvesting ^{14}C-Labelled *P. aeruginosa:*

i) The overnight culture was removed from the shaker and gently sparged with CO_2 to remove $^{14}CO_2$ (in the fume hood).

ii) The flask contents were transferred to a 250 mL centrifuge tube and spun at 10,000 RPM (15 min).

iii) After the supernatant was decanted, the pellet was washed by re-suspending in 100 mL control feed (growth medium composition shown in Table 3.2 along with 0.34 g $NaHCO_3$).

iv) Suspension was spun at 10,000 RPM (15 min) and the supernatant decanted.

v) The pellet was re-suspended in ≈15 mL of control feed and stored in a 38 mL serum bottle at 4°C.

3.5.1.2 **Isotherm Experiments**

1. Calculating and diluting ^{14}C-Labelled *P. aeruginosa:*

A suspension of ≈$1 \cdot 10^8$ cells/mL was used. 2 mL of the ^{14}C-labelled *P. aeruginosa* suspension were washed by centrifuging (13,000 RPM for 5 min), decanting supernatant, re-suspended the pellet in 2 mL control medium (this procedure was repeated twice). A 10^{-1} and 10^{-2} dilution was prepared and then a Petroff-Hauser counting chamber was used to count the number of cells/mL in the 10^{-2} dilution. 1.29 mL of the 10^{-1} dilution (≈$3.5 \cdot 10^8$ cells/mL) was added to 3.2 mL control medium to give ≈4.5 mL of $1 \cdot 10^8$ cells/mL.

2. Isotherm Procedure:

Six different amounts of the support materials (activated carbon or synthetic ion exchange resin) were weighed out according to the procedure of van Vliet et al. (1980) and added to 8 mL capacity screw cap glass vials in ≈13 mg increments (13-80 mg/vial). Then 3.8 mL of the control medium and 200 µL of the $1 \cdot 10^8$ cells/mL *P. aeruginosa* suspension were added to the vials. A vial without support material was also prepared. The vials were capped tightly and taped flat on a desk top shaker. The shaker was calibrated at 200 RPM and the vials were incubated at room temperature for 2 (or 4) h.

3. Counting the Non-adsorbed Cells:

Each vial was removed from the shaker and positioned upright to allow the support material to settle to the bottom.

Then, 2 - 1.3 mL aliquots were removed from each vial with a Pasteur pipette and transferred to micro-centrifuge (m.c.) tubes. The m.c. tubes were spun (2,000 RPM for 5 min) then 1000 µL portions were removed with an Eppendorf pipette and transferred to scintillation vials along with 10 mL aqueous counting scintillant (ACS - Amersham Corp., Illinois). The vials were counted for 2 min and the background (usually ≈25 dpm) subtracted. Vials that gave counts <100 dpm were re-counted for 10 min each.

4. Calculating Specific Activity of the ^{14}C-Labelled *P. aeruginosa* suspension:

The counts from the vials that contained no support materials were used to get an estimate of the original number of cells in the vials containing support materials. For example: 200 µL of $1 \cdot 10^8$ cells/mL added to 3.8 mL control feed (4 mL total) = $5 \cdot 10^6$ cells/mL. Counts of 2 - 1000 µL aliquots gave an average radioactivity of 385 dpm. Therefore:

$$\text{specific activity} = \frac{5 \cdot 10^6 \text{ cells/mL}}{385 \text{ dpm/mL}} = 1300 \text{ cells/dpm.}$$

The number of cells in the vial containing support material was determined by multiplying the specific activity by the counts (dpm) recorded for each vial.

3.5.2 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to observe the support material surfaces (activated carbon and synthetic ion exchange resins) in the absence and presence of biological growth. The SEM procedure was performed by Edith Schwalt, Faculty of Dentistry, University of Alberta, Edmonton, Alberta.

The following procedure was used for sample preparation and SEM viewing:

1. Support materials were fixed overnight in 2.5% glutaraldehyde in Milloning's Buffer (see Table 3.4).
2. Samples were rinsed 3 times (15 min each) with fresh Milloning's Buffer.
3. Samples were dehydrated in a series of ethanol solutions for 15 min each (50, 70, 80, 90% ethanol solutions).
4. Samples were dehydrated 3 times (10 min each) in fresh 100% ethanol.
5. Samples were critical point dried in a See Vac Inc. Critical Point Dryer (Florida, U.S.) with liquid CO_2.
6. Samples were mounted on aluminum stubs with silver conductive glue and dried overnight in a dessicator.
7. Samples were sputter coated with gold (75-150 nm thickness) in an Edwards Sputter Coater (Model S150B).
8. Samples were examined and photographed in a Philips SEM (Model 505).

Table 3.4 Milloning's Buffer

Chemical	Amount per Litre Distilled Water
$NaH_2PO_4 \cdot H_2O$	16.8 g
NaOH	3.86 g
Glucose	5.4 g
$CaCl_2$	0.05 g

4.0 SOLVENT EXTRACTION AND ANAEROBIC TREATMENT OF COAL LIQUEFACTION WASTEWATER

Solvent extraction was investigated as pretreatment for a (phenolic) coal liquefaction wastewater from the 544 metric tonne/day "H-coal" pilot plant located in Catlettsburg, Kentucky. The objective of extraction was the selective removal of non-phenolic organics and the selective control of the biodegradable phenolics concentration. This was achieved by adjusting the pH of the coal liquefaction wastewater prior to extraction. The extracted wastewater was then neutralized to render it amenable to biological treatment and eventually fed to anaerobic bacteria. The potential for removal of biodegradable phenolics was monitored. The treatment protocol used on the H-coal wastewater is shown in Table 4.1.

An analysis of the major chemical constituents in the H-coal wastewater was provided in Table 2.5 by Fedorak and Hrudey (1986a). The potential for selective solvent extraction of the wastewater was the first objective addressed.

4.1 Solvent Extraction

4.1.1 Introduction

Solvent extraction involves the partitioning of a solute between two immiscible liquid phases. In a situation where the solute is initially dissolved in one phase (e.g. an aqueous solution), it can be more or less completely transferred to the second phase, which is commonly an organic solvent. The removal of organic solutes from

Table 4.1 Treatment Protocol for H-coal Liquefaction Wastewater

Process	Objective
Centrifugation	Solids removal
Selective base addition[1]	Elevate wastewater pH
Solvent extraction[2]	Phenolic and organic removal
Selective acid addition[3]	Neutralization
Anaerobic treatment	Phenolic and organic fermentation to methane
Air stripping	Solvent removal

[1] NaOH.
[2] Diisopropyl ether.
[3] HCl.

industrial wastewaters by solvent extraction has been applied principally for the recovery of phenolic compounds (Mackay and Medir, 1983). The equilibrium behavior of an organic solute in a water-solvent system will be discussed for phenol.

4.1.2 Phase Equilibrium Principles

The equilibrium distribution coefficient, or the ratio of solute concentration in an organic solvent phase (s) and a water phase (w), is determined as a direct consequence of the thermodynamic requirements for equilibrium in the solute-water-solvent system. This distribution can be determined.

The partial molal free energy of the solute in water, G_w, can be represented by:

$$G_w = G^o_w + RT[\ln(a_w)] \qquad \text{(Eqn. 4-1)}$$

where G^o_w is the solute standard free energy and a_w is the solute activity in water. R is the Universal Gas Constant and T is the absolute temperature (°K) of the liquid.

Similarly, the partial molal free energy of the solute in an organic solvent, G_s, can be represented by:

$$G_s = G^o_s + RT[\ln(a_s)] \qquad \text{(Eqn. 4-2)}$$

where G^o_s is the solute standard free energy and a_s is the solute activity in the organic solvent.

For equilibrium to exist between the water and solvent phase we must have $G_w = G_s$. At constant temperature and pressure, it follows that:

$$G^o_w + RT[\ln(a_w)] = G^o_s + RT[\ln(a_s)]. \tag{Eqn. 4-3}$$

Rearranging equation (4-3):

$$\ln[\frac{a_s}{a_w}] = [G^o_w - G^o_s]/RT. \tag{Eqn. 4-4}$$

However, both G^o_w and G^o_s are constants for a given solute in the two liquids. Hence:

$$\ln[\frac{a_s}{a_w}] = \text{constant} \tag{Eqn. 4-5}$$

and therefore,

$$\frac{a_s}{a_w} = K. \tag{Eqn. 4-6}$$

Equation (4-6) is a mathematical statement of the "Nernst distribution law" and holds true for a given temperature (Maron and Lando, 1974). As the solute concentration approaches infinite dilution in both phases, the solute activity can be approximated by concentration (c) and equation (4-6) becomes:

$$\frac{c_s}{c_w} = K_c. \tag{Eqn. 4-7}$$

The distribution coefficient (K_c) in equation (4-7) is expressed in concentration units (e.g. $\frac{\text{moles/L}}{\text{moles/L}}$ or $\frac{\text{mg/L}}{\text{mg/L}}$). Distribution coefficients are commonly expressed in mole fraction units (K_x) and are related to K_c as follows (Mackay and Medir, 1983):

$$K_x = K_c[\frac{v_s}{v_w}] \tag{Eqn. 4-8}$$

where v_i is the molar volume (liquid density divided by the molecular weight; L/mole) of the water or solvent phase. Furthermore, K_x is directly related to the activity coefficients of the solute in the solvent ($¥_s$) and the solute in the water ($¥_w$) phase by:

$$K_x = \frac{¥_w}{¥_s}. \qquad \text{(Eqn. 4-9)}$$

The activity coefficient of phenol in water at infinite dilution has been shown to be approximately equal to 50.4 (Kiezyk and Mackay, 1973). The process of solvent selection for removal of phenol from aqueous solution involves choosing a solvent with low $¥_s$ values. This results in a high distribution coefficient (K_x) and allows high water to solvent volume ratios.

4.1.3 **Solute Ionization Effects**

Some organic solutes will ionize in aqueous solution as weak acids. This introduces a pH effect on the extraction. Consider, for example, phenol in water with an ionization constant:

$$K_a = \frac{[H^+]_w[C_6H_5O^-]_w}{[C_6H_5OH]_w} \qquad \text{(Eqn. 4-10)}$$

according to the reaction:

$$C_6H_5OH <===> H^+ + C_6H_5O^-. \qquad \text{(Eqn. 4-11)}$$

The subscript w denotes the aqueous phase concentration.

The equilibrium distribution coefficient for phenol in a water - solvent mixture can be demonstrated from equation (4-7):

$$K_c = \frac{[C_6H_5OH]_s}{[C_6H_5OH]_w}. \qquad \text{(Eqn. 4-12)}$$

However, it is more meaningful to describe a different term, the distribution ratio D (Christian, 1980), which is the ratio of the concentrations of all species of phenol in each phase. Total phenol species in the solvent phase is given by:

$$[C_6H_5OH]_s. \qquad \text{(Eqn. 4-13)}$$

The total phenol species in the water phase (accounting for the ionization) is given by:

$$[C_6H_5OH]_w + [C_6H_5O^-]_w. \qquad \text{(Eqn. 4-14)}$$

Hence, D is expressed as:

$$D = \frac{[C_6H_5OH]_s}{[C_6H_5OH]_w + [C_6H_5O^-]_w}. \qquad \text{(Eqn. 4-15)}$$

From equation (4-12):

$$[C_6H_5OH]_s = K_c\,[C_6H_5OH]_w. \qquad \text{(Eqn. 4-16)}$$

From equation (4-10):

$$[C_6H_5O^-]_w = \frac{K_a\,[C_6H_5OH]_w}{[H^+]_w}. \qquad \text{(Eqn. 4-17)}$$

Substitution of equations (4-16) and (4-17) into (4-15) gives:

$$D = \frac{K_c\,[C_6H_5OH]_w}{[C_6H_5OH]_w + K_a\,[C_6H_5OH]_w/[H^+]_w}, \qquad \text{(Eqn. 4-18)}$$

which reduces to:

$$D = \frac{K_c}{1 + K_a/[H^+]_w}. \qquad \text{(Eqn. 4-19)}$$

Substitution of the acid dissociation constant (K_a) value for phenol ($10^{-10.0}$ at 25°C; Windholz, 1983) gives:

$$D = \frac{K_c}{1 + 10^{-10.0}/[H^+]} \qquad \text{(Eqn. 4-20)}$$

Equation (4-20) predicts that when $[H^+]_w >> 10^{-10.0}$ (i.e. pH << 10.0), D is nearly equal to K_c. If K_c is large, phenol will be extracted into the solvent phase. If on the other hand, $[H^+]_w << 10^{-10.0}$ (i.e. pH >> 10.0), equation (4-20) reduces to:

$$D = \frac{K_c [H^+]_w}{10^{-10.0}} \qquad \text{(Eqn. 4-21)}$$

D will be smaller as a result and phenol will remain in the water phase.

Hence it can be seen that phenol concentrations in water can be selectively controlled during solvent extraction. An excellent potential exists for applying this rationale to remove excess phenol from the H-coal wastewater then feeding the extracted wastewater to anaerobic bacteria for conversion of the remaining biodegradable organics to methane.

4.1.4 Solvent Extraction Potential of High Strength Phenol Wastewater

Industrial wastewaters containing high phenol concentrations (e.g. >5,000 mg/L) can be extracted with a suitable solvent (i.e. low phenol activity coefficient, $¥_s$) at high wastewater:solvent volume ratios. Also, the desired aqueous phenol concentration after extraction can be selectively controlled by adjusting the wastewater pH prior to extraction. The advantage of this rationale is that wastewater phenol concentrations can be adjusted to a level that is amenable to anaerobic biological treatment. The combination of solvent extraction followed by anaerobic treatment has the potential to completely eliminate high phenol levels from the wastewater. Also, prior solvent extraction removal of low polarity or non-polar organics that may be non-biodegradable and/or inhibitory to bacteria will allow subsequent anaerobic treatment to function more effectively. Many of these organics may be representative of the background matrix that is typically associated with phenolic wastewaters. The solvent extraction behavior of substituted phenol compounds (e.g. o-, m- , and p-cresol) which are present in some phenolic industrial wastewaters will be generally similar to phenol because, like phenol, they are weak acids.

4.1.5 **Solvent Selection**

The ideal solvent for extraction of high strength phenol wastewaters is one that possesses a low phenol activity coefficient (¥$_s$) to allow a high wastewater:solvent flow rate, an important economic consideration.

Kiezyk and Mackay (1973) stated that polar hydrogen bonding solvents (e.g. ethers, ketones, esters, and alcohols) are preferred over hydrocarbon and chlorinated hyrdocarbon solvents because of their higher distribution coefficients for phenol (i.e. lower ¥$_s$). Another reason for their preference is because the hydrogen bonding solvents are less toxic to microorganisms, and, are more susceptible to biodegradation. This has important consequences for the intended purpose of extraction, i.e. as a pretreatment to anaerobic biological treatment. A drawback of the preferred solvents is their increased solubilities in water. Hence, the potential exists for solvent toxicity or excess organic loading to the anaerobic process.

Of the preferred solvents for phenol extraction, diisopropyl ether (DIPE) and methyl isobutyl ketone (MIBK) have been used. Both are commercially employed for phenol recovery from industrial wastewaters (Medzadourian et al., 1983). Several discussions have been presented on the relative merits of each solvent for the extraction of phenol and substituted phenol compounds (Senetar and King, 1986; Medzadourian et al., 1983; Greminger et al., 1982). Table 4.2 lists the physical properties and experimental distribution characteristics of phenol at low pH and high dilution for DIPE and MIBK.

Based on the small scale of extraction experiments planned it was decided that DIPE would be used. MIBK is more effective than DIPE for phenol extraction based on the higher experimental K_c values observed in Table 4.2. However, DIPE is less soluble in water than MIBK and, therefore, less of a concern with respect to toxicity or excess substrate loading to the anaerobic process.

4.1.6 **Extraction Procedure**

Prior to extraction, the wastewater was treated to remove solids. Wastewater samples were centrifuged for 20 min at 10,000 RPM with a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Du Pont Instruments).

Individual portions of the centrifuged wastewater were adjusted to select pH with 1 N (or 5 N) NaOH. The pH-adjusted wastewater (250 mL) was transferred by graduated cylinder to a

Table 4.2 Physical Properties of DIPE and MIBK and Experimental Distribution Characteristics of Phenol at Low pH and High Dilution

Parameter	DIPE	MIBK
Molecular weight	102.17	100.16
Melting point (°C)	-85.5(1)	-84.0(1)
Boiling point (°C)	68.3(1)	116.5(1)
Density @ 25°C (g/mL)	0.7182(1)	0.7961(1)
Water solubility (% wt.)	1.2 @ 20°C(1) 0.9 @ 25°C(2)	1.7 @ 25 °C(1)
Experimental γ_s^∞	0.31(3) 0.222(4)	0.105(3)
Experimental K_c $(mg \cdot L^{-1})/(mg \cdot L^{-1})$	29 @ 25°C(2) 26.3 @ 25°C(5)	80 @ 30°C(3) 67.7 @ 30°C(5)

γ_s^∞ = Solvent activity coefficient of phenol.

K_c = Concentration based equilibrium distribution coefficient for phenol.

(1) Riddick and Bunger, 1970.
(2) Mackay and Medir, 1983.
(3) Greminger et al., 1982.
(4) Kiezyk and Mackay, 1973.
(5) Senetar and King, 1986.

separatory funnel (500 mL capacity). DIPE (25 mL) was added to the funnel using a pipette. The funnel was shaken for 2 min and set on a rack to allow phase separation under quiescent conditions for 13 min.

The wastewater and spent DIPE fractions were withdrawn into separate glass beakers. The funnel was rinsed with a 50 mL solution of 15% acetone in hexane (2 times) and 50 mL deionized distilled water (3 times) and allowed to drain for several minutes. This procedure aided the removal of a colloidal emulsion that had formed in the solvent phase (near the interface) and did not drain properly from the funnel. The extracted wastewater was transferred back to the funnel along with 25 mL of fresh DIPE and the mixing, separation, and rinse procedure repeated twice more.

The extracted H-coal wastewater was then neutralized to pH 7 with 1 N (or 5 N) HCl. The total addition of NaOH and HCl to all pH-adjusted wastewater samples resulted in volume dilutions of less than 1% and the resultant solute concentration dilution effect was neglected.

The extracted wastewater samples (in open beakers) were placed in a fumehood for 16 h. This allowed DIPE to evaporate from the samples.

The pH of each sample was checked to ensure a final value between 6.9 and 7.2. The samples were stored in capped glass bottles at 4°C until further use.

4.2 Characterization of Extracted Wastewater and Solvent

Characterization of the main constituents in the un-extracted H-coal wastewater was originally provided by Fedorak and Hrudey (1986a) in Table 2.5. Wastewater from the same batch had been stored in 5 gallon containers (4°C) until used in this study. Any differences in the concentrations of constituents presented in this study with Table 2.5 are probably due to chemical changes that occurred over the period the wastewater was in cold storage (2 years). Also, different instruments (HPLC and GC) were used for the analysis and could account for small variations.

4.2.1 Solvent Extraction Recovery of Phenolics

The theoretical DIPE extraction efficiency of phenol from pure water and H-coal wastewater as a function of pH is shown in Figure 4.1. The extraction efficiency from water is shown for a single and a triple extraction using an aqueous phenol solution to DIPE solvent ratio of 10:1 (see Appendix I). An acid dissociation constant (pK_a) for phenol (10.0) and phenol distribution coefficient (weight fraction, K_d) of 36.8 were used. The extraction efficiency decreases dramatically from pH 9 to 12. This range brackets the acid dissociation constant for phenol in pure water. Higher phenol extraction efficiency occurs at pH << pK_a. Phenol will primarily be in the un-dissociated form at low pH and have a strong affinity for the DIPE solvent phase.

The actual phenol extraction efficiency from H-coal wastewater is also shown in Figure 4.1. These trials employed three successive extractions with a wastewater to solvent ratio of 10:1. The actual

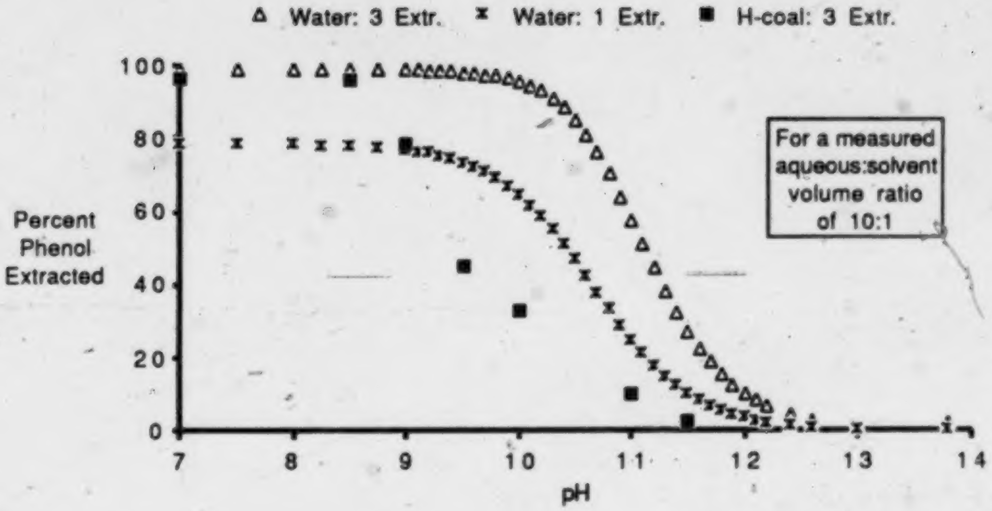

Figure 4.1 Extraction Efficiencies of Phenol from Pure Water and H-coal Wastewater with DIPE

phenol extraction efficiency was substantially lower than the theoretical extraction efficiency for three extractions. A possible explanation for the marked difference is that the theoretical extraction efficiency of phenol from pure water assumes thermodynamic equilibrium was attained (which would only occur at a solvent-water contact time = infinity). The actual extraction efficiency of phenol from the H-coal wastewater was based on a 15 min contact time (see section 4.1.6). It is likely that true equilibrium conditions did not exist during extraction of the H-coal wastewater and contributed to the lower extraction efficiency. Also, the presence of other organics and inorganics in the H-coal wastewater would reduces the activity coefficient of phenol in DIPE (γ_s) and in water (γ_w). If γ_w is reduced more than γ_s, the distribution coefficient (K_x) is lower (equation 4-9) and the resulting extraction efficiency would be lower.

The theoretical maximum phenol extracted for a single extraction is approximately 78.5%. This maximum is influenced by the water to solvent ratio. The maximum extraction efficiency would increase with a smaller water to solvent ratio (Appendix I).

The aqueous concentrations of phenol, cresols, and COD in the solvent extracted wastewater at different pH values are summarized in Table 4.3. The extraction efficiency of phenol and the cresols change rapidly in the range of their respective acid dissociation constants (see Table 4.4).

The COD values in Table 4.3 indicate the substantial reduction of wastewater organic fraction achieved by solvent extraction at lower pH values. The remaining phenol and m/p-cresol concentrations

Table 4.3 Residual Phenol, Cresols, and COD Concentrations in the H-coal Wastewater after DIPE Extraction at Various pH Values

Extraction pH	Phenol (mg/L)	m/p-Cresol (mg/L)	o-Cresol (mg/L)	COD (mg/L)
7	160	< 5	N.D.	N.A.
8.5	205	10	N.D.	2,720
9	1,050	35	N.D.	5,550
9.5	2,660	240	30	8,770
10	3,250	520	80	13,000
11	4,360	1,080	235	N.A.
11.5	4,720	1,360	355	N.A.
un-extracted	4,850	1,550	515	21,000

N.D. = Not detected.
N.A. = Not analyzed.

Table 4.4 Acid Dissociation Constants (pK_a) of Phenol and the Cresols

Compound	pKa[1]	Reference
Phenol	10.0	Windholz, 1983
m-Cresol	10.01	Weast, 1978
p-Cresol	10.17	Weast, 1978
o-Cresol	10.20	Weast, 1978

[1] @ 25°C.

account for only 47% of the measured COD at pH 9. The remaining COD would be attributed to other phenolics, non-extracted organics (e.g. volatile organic acids - see Table 2.5), and soluble DIPE. DIPE has a water solubility of 0.9% wt. (Table 4.2) - approximately 8,940 mg/L at 25°C (see Appendix II). If the worst case situation is assumed, i.e. the remaining COD fraction (53%) is solely attributed to soluble DIPE, the corresponding DIPE concentration is calculated at 1,050 mg/L (see Appendix II). This level is well below the maximum solubility.

The presence of DIPE in the extracted wastewater had to be addressed. It may have been inhibitory or, if biodegradable, its presence would add an excess substrate loading to the anaerobic process. DIPE may have to be removed from the wastewater (e.g. by stripping or distillation) before or after anaerobic treatment to improve the overall quality of the treated wastewater.

4.2.2 Extracted Wastewater Acidity and Buffer Characteristics

Several of the extracted wastewater samples and the original un-extracted wastewater were titrated to measure their buffer intensity. The titration behavior of the samples is shown in Figure 4.2. The titration curves indicate the amount of strong base added per litre of wastewater as a function of pH. An estimate of the amount of base required to raise the pH of the wastewater can be obtained. The base requirement to raise the wastewater pH to 9 is 30 meq/L (to pH 10 is 74 meq/L).

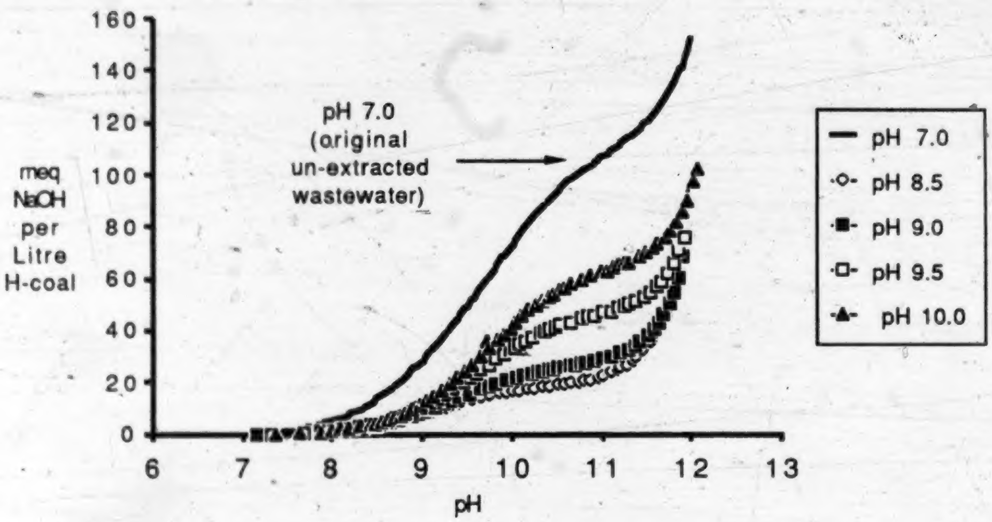

Figure 4.2 Titration Behavior of pH Selective Solvent-extracted Wastewater

Information can be obtained from the samples by plotting the slope of the titration curves versus pH, called a buffer intensity plot. The buffer intensity of a solution describes its ability to resist changes in pH from the addition of acids or bases. Water exerts significant buffering below 3 and above 11, and its contribution must be subtracted (Snoeyink and Jenkins, 1980). Buffer intensity plots for the same samples that were titrated are shown in Figure 4.3 (after elimination of buffering provided by water). The buffer intensity of the un-extracted wastewater shows a broad maxima in the pH range 9.6 to 10.2. This range agrees well with values of the observed acid dissociation constants (pK_a) for phenol and the cresols (Table 4.4) and indicates that these phenolics are partially responsible for the buffering observed in this pH range. Compounds that dissociate in water display their greatest buffering capacity when the pH of the water is equal to the compound pK_a. The presence of other weakly acidic compounds with dissociation constants in the same pH range as phenol and the cresols would also influence the buffer capacity. The buffer intensity maxima for the extracted samples decrease at the lower extraction pH values (pH 8.5 and 9, Figure 4.3). This is consistent with greater amounts of phenol, cresols, and other weak acidic compounds removed during extraction (Table 4.3).

The residual buffering observed around pH 9 for the wastewater extracted at pH 8.5 (Figure 4.3) may have been due to dihydric phenols. Dihydric phenols are more difficult to extract from solution than phenol because of their increased aqueous solubility arising from the additional hydroxyl group. Lyman et al. (1982) reported a

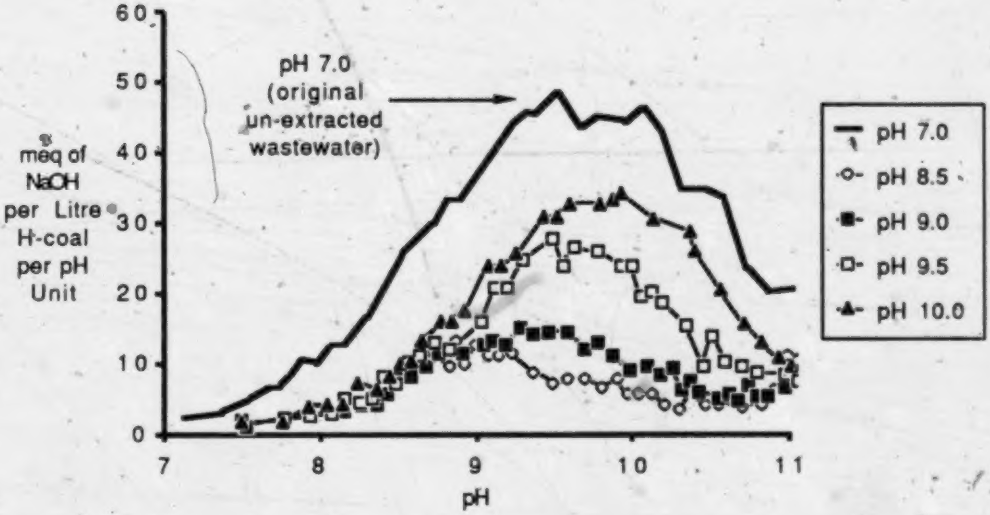

Figure 4.3 Buffer Intensity of pH Selective Solvent-extracted Wastewater (Adjusted for the Effect of Water)

measured pK_a for o-hydroxyphenol (i.e. catechol) of 9.45. The presence of catechol or other substituted dihydric phenols may have accounted for the residual buffering near this pH range.

4.2.3 GC/MS Identification of Spent DIPE Solvent

Diisopropyl ether extraction successfully reduced the levels of phenolic and other COD components in the pH 9 adjusted H-coal wastewater (Table 4.3). GC/MS was used to identify organic compounds other than the major phenolics. Concentrations of phenol (12,670 mg/L), m/p-cresol (5,050 mg/L), and o-cresol (1,720 mg/L) in the spent DIPE were calculated based on the recovery of phenolics during pH 9 extraction (see Appendix III). As a result, the spent DIPE fraction was initially back-extracted with water adjusted to pH 12 with NaOH to remove as much of the phenolics as possible.

Table 4.5 summarizes the components identified by GC/MS. Phenol, cresol, and dimethylphenols still represented a significant portion of the major components reported even after back-extraction. Aniline, one isomer of methylaniline, and methyl biphenyl were the only other major components tentatively identified and quantitated.

Methyl biphenyl is relatively insoluble in water judging from its non-polar bonding characteristics (formula: $C_6H_5\text{-}C_6H_4\text{-}CH_3$). This compound will have a stronger affinity for the organic solvent (DIPE) during extraction of aqueous H-coal wastewater, and be concentrated in the DIPE as a result (i.e. high equilibrium distribution coefficient, K_c). DIPE is less polar than water and

Table 4.5 Tentative GC/MS Identification of DIPE Extracted Compounds from H-coal Wastewater

Major Components	Estimated Level (mg/L)
Phenol	31
Cresol	34
Dimethylphenol	5
Dimethylphenol	0.8
Aniline	17
Methylaniline	4
Methylaniline	0.3
Dimethylaniline	0.2
Methyl biphenyl	4

Minor Components

Alkanes (C8, C9, C11, C15, C21, C26, C27)[1]
Trimethylcyclopent-2-eneone
C2-Phenylethanones
Trimethylphenol
1,4-Benzenediol
Methylbenzendiols
Alkylanilines
Dimethylbenzenediol
Dihydroxyphenylethanone
Tetramethylphenol
Methoxypropenyl phenol
Dimethylbenzenedimethanthiol
Methyl-t-butylbenzenemethanone
Hexadecanol
Methylhexadecane

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[1] Number of carbons in the alkane chain, e.g. C11 = 11 carbons.

aqueous methyl biphenyl concentrations would be expected to be very low after extraction.

Aniline (chemical formula: C_6H_5-NH_2) and methylaniline (chemical formula: C_6H_5-NH-CH_3) are more soluble in water than methyl biphenyl. The hydrogen bonding feature of the nitrogen atom (-NH_2) in aniline and methylaniline is polar and accounts for the increased solubility (compared to methyl biphenyl). Both aniline compounds are weak bases and accept a proton from water in a reversible acid-base reaction. The acid-base reaction for aniline is:

$$C_6H_5\text{-}NH_2 + H_2O <\!\!==\!> C_6H_5\text{-}NH_3^+ + OH^- . \qquad \text{(Eqn. 4-22)}$$

The base dissociation constant (pK_b) for aniline is 9.37 at 25°C (Weast, 1978). At pH values less than 4.63 (i.e. less than 14 - 9.37) aniline will exhibit acidity. Likewise at pH values greater than 4.63, aniline will be present in the neutral form and more susceptible to be solvent extracted from aqueous solution. N-methylaniline (pK_b = 9.15) behaves similar to aniline.

The host of other minor components reported indicates the wide variety of organics present. Alkanes (containing 9 to 27 chain carbons) and substituted benzenes represented many of these components.

4.3 Batch Fermentation Studies

An initial set of serum bottle (batch) assays were performed to test the anaerobic biodegradability of the extracted H-coal wastewater. The biological activity of the cultures was observed by monitoring the disappearance of phenol (and cresols) and the periodic measurement of the methane concentration in the bottle headspace.

The purpose of the batch assays was:

i) To test the potential of anaerobic bacteria to biodegrade and remove phenol and the cresols from the extracted wastewater (by monitoring disappearance of the phenolics); and

ii) To observe the biological inhibition potential of the various pH extracted wastewaters (by comparing the rate of biological phenolic removal between cultures that received extracted wastewater and cultures that received a synthetic phenolic solution).

The source of inoculum for the assays was a combination of domestic anaerobic sludge (90% v/v) and sludge acclimated to phenol (10% v/v). The acclimated sludge had been previously maintained on phenol as the sole carbon source in a laboratory fermentation vessel.

4.3.1 Procedures

Two sets of assays were performed on the extracted wastewater. Assay I tested wastewaters that were extracted at pH values of 7, 8.5, 10, and 11.5 and compared them to a synthetic control culture. Assay II tested wastewaters extracted at pH values of 8.5, 9, 9.5, and 10 and compared them to a synthetic control culture. The synthetic control cultures received phenolics only. In addition, a culture that received distilled water only was set up to act as a blank. All cultures were set up in triplicate and incubated at 37°C for 6 weeks.

In order to have a similar basis for comparing the activities of the different cultures, it was decided to give them all the same approximate phenolic concentration at the beginning of each assay. Assay I had some cultures supplemented with synthetic phenolics to achieve approximate concentrations of 500 and 750 mg/L total phenolics. Assay II also had some cultures supplemented with synthetic phenolics to give them all approximate concentrations of 750 mg/L total phenolics. Table 4.6 shows the constituents (and volumes) that were added to 119 mL serum bottles for both assays.

4.3.2 **Results and Discussion**

All incubated cultures from both assays developed the microbial diversity to convert phenol, m-cresol, and p-cresol to methane gas. Figure 4.4 demonstrates biological phenol removal from the cultures initially containing 750 mg/L total phenolics from Assay I. Similarly, Figure 4.5 shows biological m/p-cresol removal for the same set of cultures. The methane concentrations (%) in the cultures headspace at day 22 and 32 are shown in Table 4.7. Biological phenolic removal from all cultures in both bioassays and methane concentrations observed in Assay II are tabulated in Appendix IV.

Biological degradation of o-cresol was not observed over the 42 day incubation period (Appendix IX). It appears that during the early days of incubation in both assays the o-cresol levels decreased significantly from the initial concentration at day 0. o-Cresol concentrations did not change very much for the remainder of the incubation perod. Sorption of o-cresol to sludge (organics) may have accounted for the observed decreases during the initial stages of

Table 4.6 Serum Bottle Constituent Volumes Batch Fermentation

Cutlure[1]	Synthetic Phenoi Solution[2] (mL)	Distilled Water (mL)	pH Extracted H-coal (mL)
Assay I:			
Extr. pH 7 (500)	2.9	3.1	4.0
" (750)[3]	4.3	0	6.0
Extr. pH 8.5 (500)	2.8	3.1	4.0
" (750)[4]	4.2	0	6.0
Extr. pH 10 (500)	1.0	5.0	4.0
" (750)	1.5	2.5	6.0
Extr. pH 11.5 (500)	0	6.0	4.0
" (750)	0	4.0	6.0
Blank	0	10.0	0
Synthetic (500)	2.9	7.1	0
" (750)	4.4	5.6	0
Assay II:			
Extr. pH 8.5 (750)[3]	4.3	0	6.0
" 9 (750)	3.7	0.3	6.0
" 9.5 (750)	2.4	1.6	6.0
" 10 (750)	1.3	2.7	6.0
Blank	0	10.0	0
Synthetic (750)	4.5	5.5	0

---

[1] Numbers in the brackets represent approximate total phenolic concentration (mg/L) at the beginning of the assay.

[2] Contained 9,000 mg/L phenol; 600 mg/L o-cresol; 900 mg/L m-cresol; and 900 mg/L p-cresol.

[3] Total liquid volume of 50.3 mL.

[4] Total liquid volume of 50.2 mL.

Note: 35 mL of sludge inoculum and 5 mL of nutrient medium were added to give a total liquid volume of 50 mL (unless otherwise noted) for all cultures.

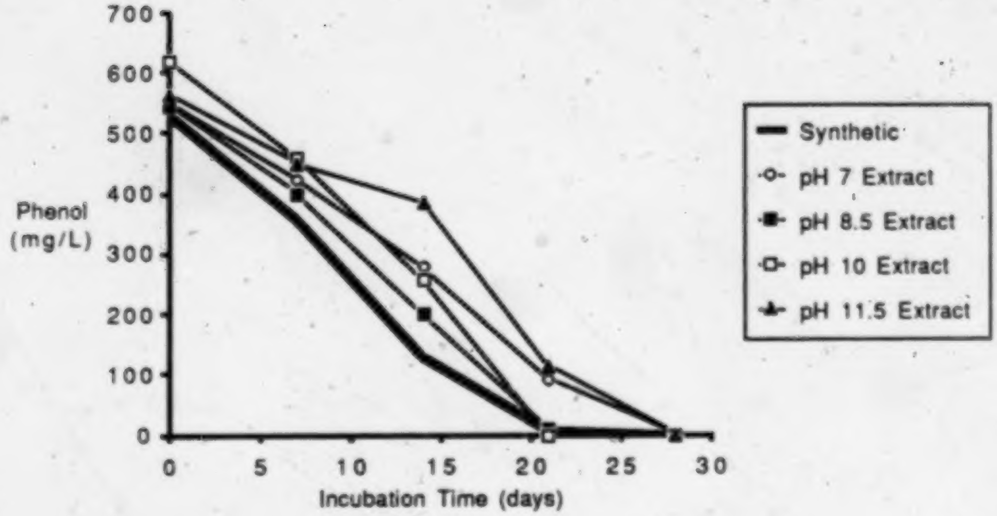

Figure 4.4 Assay I - Batch Fermentation of Phenol in Extracted H-coal Wastewater (750 mg/L Total Phenolics)

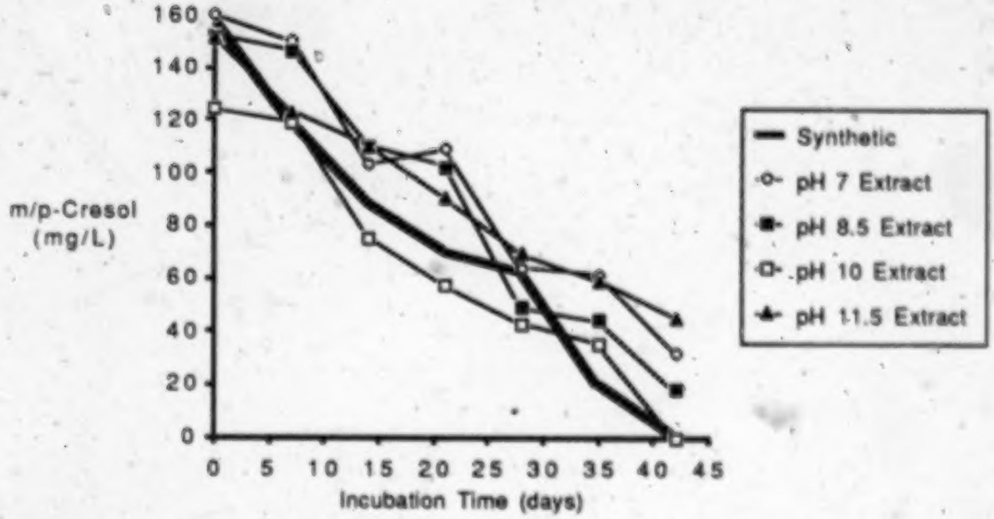

Figure 4.5 Assay I - Batch Fermentation of m/p-Cresol in Extracted H-coal Wastewater (750 mg/L Total Phenolics)

Table 4.7 Assay I - Percent Methane in Culture Headspace

	Incubation Day 22	Incubation Day 32
	Methane Concentration (%)	
Culture[1]:		
Extr. pH 7 (500)	28.7	28.4
" (750)	-	33.9
Extr. pH 8.5 (500)	27.5	27.5
" (750)	28.8	32.4
Extr. pH 10 (500)	30.1	27.9
" (750)	32.6	31.1
Extr. pH 11.5 (500)	27.4	27.5
" (750)	32.7	31.1
Blank	11.0	14.5
Synthetic (500)	26.0	26.6
" (750)	28.6	29.6

[1] Numbers in the brackets represent approximate total phenolic concentration (mg/L) at the beginning of the assay.

incubation. All cultures had a large amount of sludge innocula (35 mL sludge per 50 total liquid volume - see Table 4.6). Boyd (1982) found that cresols were less soluble in water and had larger octanol/water partition coefficients than phenol, and cresols sorbed to soils to a greater degree than phenol. The fact that aqueous o-cresol persisted for the duration of the incubation is not unusual. Fedorak and Hrudey (1986a) and Boyd et al. (1983) reported similar findings.

Phenol and m/p-cresol degradation was not substantially inhibited in any of the cultures containing DIPE extracted wastewater. This is demonstrated by the similar rates of phenol (Figure 4.4) and m/p-cresol (Figure 4.5) removal, respectively, for the different cultures compared to the culture containing the synthetic phenolic mixture.

Fedorak and Hrudey (1985) reported that batch anaerobic methanogenesis of the same H-coal wastewater (un-extracted) in serum bottles was inhibited at percent by volume concentrations (i.e. volume of H-coal/total liquid volume) greater than 6%. This corresponded to an initial total phenolic concentration of 428 mg/L. This concentration represented the dilution of original un-extracted wastewater. The current study was able to demonstrate negligible inhibition at double the percent by volume concentrations of H-coal (i.e. 12% in the cultures receiving 750 mg/L total phenolics). The 750 mg/L total phenolic level is closer to the level which inhibits phenolic degradation in batch cultures (Fedorak and Hrudey, 1984). Despite these stresses, biological phenolic removal from the cultures receiving H-coal wastewater extracted at pH 8.5 or 10 was similar to the culture that received the synthetic mixture. This suggests that the inhibitors previously observed by Fedorak and Hrudey (1985) were extracted in the current study and demonstrates the benefits of using solvent extraction as pretreatment to anaerobic treatment.

Data in Figure 4.4 and 4.5 suggest that the wastewater extracted at pH 7 and 11.5 may have been slightly inhibitory compared with the synthetic mixture. However, consideration of the methane concentrations at day 32 (Table 4.7) indicates the effect was minimal compared with the severe inhibition of methanogenesis observed by Fedorak and Hrudey (1985) at H-coal volume concentrations of 8%. Methane concentrations reported in Table 4.7 are slightly higher in the cultures containing extracted wastewater compared with the synthetic mixture. Because the H-coal wastewater contains non-extractable volatile organic acids (Table 2.5) which were not included in the synthetic mixture, the latter would be expected to yield less methane.

Results of the solvent extraction trials and anaerobic batch fermentation assays demonstrate complete removal of phenol and m/p-cresol may be possible. Solvent extraction proved successful in minimizing (or removing) unknown biological inhibitors from the wastewater, and, removing excess phenolics prior to anaerobic treatment.

This evaluation provided the basis for developing experimental semicontinuous anaerobic cultures for treating solvent extracted H-coal liquefaction wastewater without the need for dilution. Solvent extraction of the wastewater at a pH of 9 or less would produce a wastewater:

i) relatively free of unknown biological inhibitors,

ii) with phenolic concentrations that would not be inhibitory to acclimated anaerobic bacteria, and

iii) with o-cresol concentrations present at less than detection level limits (o-cresol is difficult to biodegrade anaerobically and therefore its complete removal during extraction would be preferred).

4.4 Semicontinuous Fermentation Studies

Semicontinuous (draw and feed) experiments were performed to further assess the potential of anaerobic biodegradability of phenolics in the extracted H-coal wastewater. The draw and feed technique, described in Fedorak (1984), allows the simple and direct evaluation of biological performance under a variety of organic loading conditions. This technique provides a long mean cell residence time while maintaining a constant HRT by settling the biological solids prior to removing supernatant from the cultures. This procedure allows the demonstration of mean cell residence times that would occur in attached-growth systems.

The extracted wastewater was supplemented with nutrients and initially fed to anaerobic cultures at two different organic loading conditions. The cultures were monitored for biological removal of phenol and m/p-cresol, and steady-state volumetric methane production under these conditions. The source of inoculum for this experiment was domestic anaerobic sludge unacclimated to phenolic compounds.

4.4.1 **Procedures**

All conditions tested were performed with triplicate cultures and incubated at 37°C. Initially, 45 mL of domestic anaerobic sludge and 5 mL of a growth medium were added to 119 mL flushed serum bottles and incubated 24 hours prior to being fed the extracted H-coal wastewater.

The feed solution was prepared by anaerobically dispensing 90 mL of the extracted wastewater along with 10 mL of the nutrient growth medium into 119 mL flushed serum bottles, which were then capped and sealed. A 1 mL portion of sulfide solution (Table 3.1) was added by needle and syringe to ensure a reduced feed. A blank feed solution using tap water was set up in similar fashion.

A draw and feed procedure was started with the cultures according to the schedule in Table 4.8. Cultures that received extracted H-coal wastewater were fed semicontinuously for 250 days before the experiment was terminated. The blank culture was fed for 72 days in order to get a measure of the methane potential in the 45 mL inoculum. The different conditions tested with the extracted H-coal wastewater were as follows:

i) Phase I (day 1-73): The feed solution during this period was pH 8.5 extracted H-coal wastewater. The cultures were drawn and fed daily.

Table 4.8 Summary of Semicontinuous Cultures at Startup

Draw and Feed Volume (mL/day)[1]	HRT (days)[2]	Feed Solution[3]
3	16.7	Tap water (blank)
3	16.7	Extracted H-coal Wastewater
2	25	Extracted H-coal Wastewater

[1] Fed to anaerobic cultures of 50 mL total liquid volume.
[2] Hydraulic retention time.
[3] Supplemented with 10% by volume nutrient growth medium.

ii) Phase II (day 73-97): The feed solution was a 1:1 mixture of pH 8.5 and 9 extracted wastewaters. The cultures were drawn and fed daily.

iii) Phase III (day 97-176): The feed solution was pH 9 extracted wastewater. The cultures were drawn and fed every second day. On day 109 activated carbon was introduced into the cultures. Each individual culture (50 mL) was used to create two new cultures of 25 mL liquid volume each. The new cultures were administered one half the original draw and feed volume shown in Table 4.8 to maintain the same HRT for each triplicate set. Setup of the new cultures was accomplished by anaerobic transfer with a flushed 50 mL plastic syringe and 18G x 3.8 cm needle (B-D) to flushed 58 mL serum bottles. One of the duplicate serum bottles contained 26.4 mg (average dose) of 75-150 µm Filtrasorb® 300 activated carbon (Calgon Corp.).

iv) Phase IV (day 176-219): The feed solution was pH 9 extracted wastewater. All cultures were initially fed individually on demand and eventually brought up to an equivalent HRT of 12.5 days.

v) Phase V (day 220-250): The feed solution was pH 9 extracted wastewater. All cultures were maintained at a HRT of 12.5 days. The cultures were drawn and fed daily.

At no time during the experiment were any of the cultures supplemented with fresh or phenolic acclimated sludge.

4.4.2 Results and Discussion

Data from the cultures maintained at a HRT of 16.7 d will be presented and discussed here as this situation represents the highest stress condition tested. Data from the cultures maintained at 25 d HRT is presented in Appendix V and is discussed in this section where applicable.

Phase I and II (day 1-97)

Initial semicontinuous draw and feeding of pH 8.5 extract to triplicate cultures maintained at 16.7 d HRT exhibited excellent removal of phenol. Figure 4.6 demonstrates the influent and effluent phenol concentrations during Phase I. Effluent phenol concentrations were maintained below 3 mg/L for the 73 day period. Upon switching to a 1:1 mixture of pH 8.5 and 9 extracted wastewaters, which reflected an increase in influent phenol from 220 to 520 mg/L, an immediate response in the effluent was observed. Effluent phenol increased to 30 mg/L, however, within 8 days it eventually decreased to levels that fluctuated between 1 and 13 mg/L.

Response of the cultures to m- and p-cresol is shown in Figure 4.7. Capacity for m/p-cresol removal was very poor in general. Phase I influent concentrations averaged 6 mg/L and m/p-cresol appeared in the effluent (4 mg/L) only after 20 days operation. Analysis of the effluent collected from day 30 to 60 revealed m-cresol as the dominant methylphenol present. Fedorak and Hrudey (1986a) performed semicontinuous draw and feed studies with dilutions of the same H-coal wastewater (un-extracted). They observed m-cresol as the first phenolic to appear in the effluent and its concentration

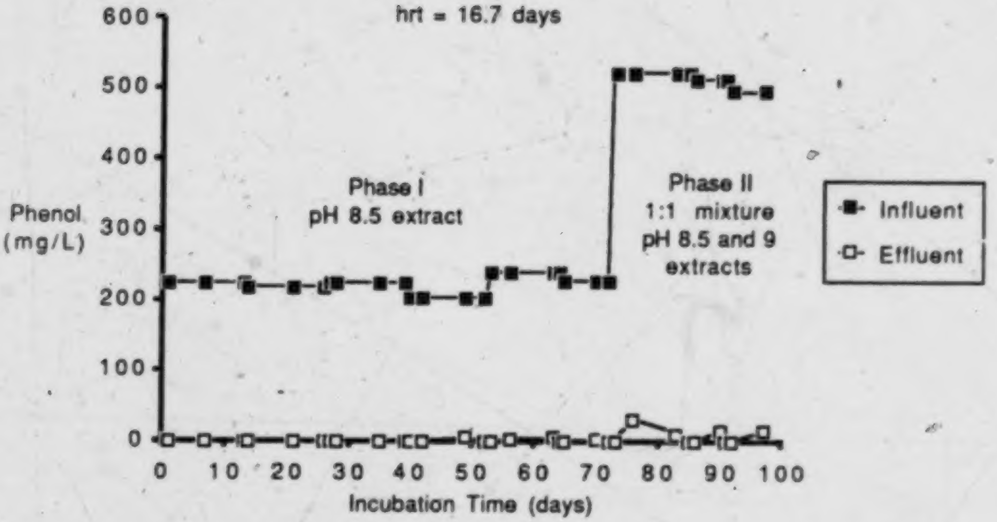

Figure 4.6 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater - Phase I and II

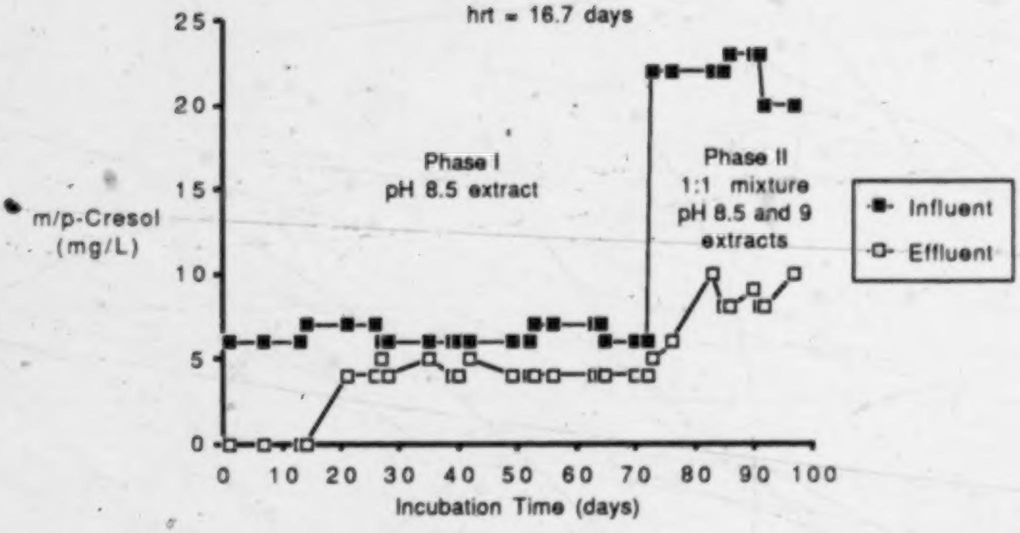

Figure 4.7 Semicontinuous Fermentation of m/p-Cresol in Extracted H-coal Wastewater - Phase I and II

appeared to follow a simple washout curve suggesting that its degradation stopped suddenly.

Characteristics of effluent m/p-cresol during Phase I of this study did not follow the same phenomena observed by Fedorak and Hrudey (1986a). m/p-Cresol did suddenly appear in the effluent at 4 mg/L after 20 days feeding, however, after 70 days it was still maintained at 4 mg/L. It is evident that some mechanism was responsible for the partial m/p-cresol removal (i.e. biodegradation) during this period. Upon switching to the 1:1 mixture of pH 8.5 and 9 extracted wastewaters, which reflected an increase in influent m/p-cresol of 6 to 22 mg/L, effluent concentrations increased immediately. The effluent m/p-cresol concentration was analyzed at this point to determine if the step increase inhibited the cultures ability to remove m/p-cresol (see Appendix VI). Although effluent concentrations increased immediately, the increase did not follow a simple washout curve suggesting that removal mechanisms were still operating.

Figure 4.8 shows the volumetric methane production for both triplicate sets of cultures (16.7 and 25 d HRT) at the incubation temperature of 37°C and 1 atm. The amount of influent COD converted to methane gas during Phase I and II is shown in Table 4.9 (see Appendix VI). The data from Table 4.9 indicate that less influent COD was removed and converted to methane at higher organic loading conditions (Phase II). The fact that near complete phenol removal was observed in Phase I and II (see Figure 4.6) appears to indicate that organics (other than phenol) account for the COD not removed as methane. The calculated amount of phenol as

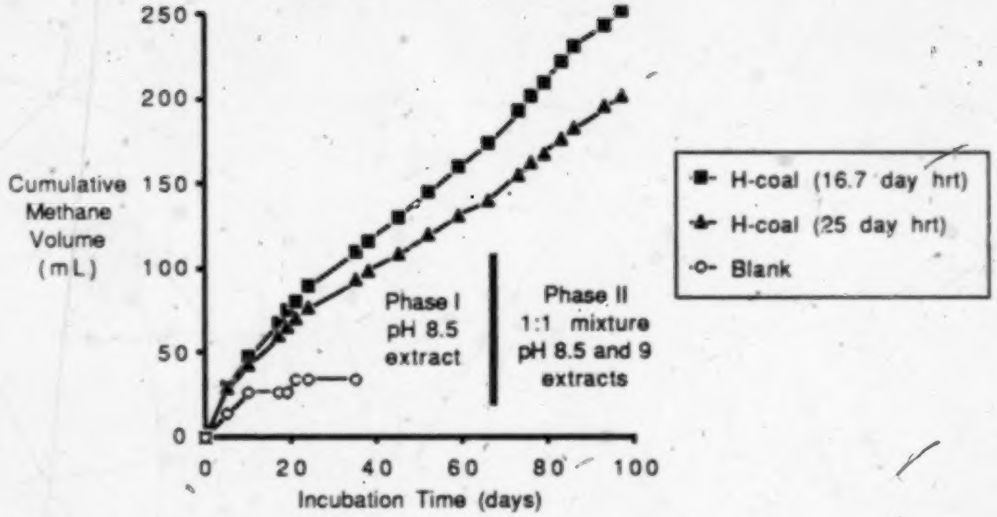

Figure 4.8 Methane Production during Semicontinuous Fermentation of Extracted H-coal Wastewater - Phase I and II

Table 4.9 Methane Generation Efficiency Based on COD Equivalence - Phases I and II

Culture	CH_4 Produced (mL/d)[1]	COD of CH_4 (mg/d)[2]	COD Fed (mg/d)[3]	COD Removed as CH_4 (%)
Phase I:				
25 day HRT	1.5	3.8	4.9	78
16.7 day HRT	2.0	5.0	7.4	68
Phase II:				
25 day HRT	2.1	5.3	7.4	72
16.7 day HRT	2.7	6.8	11.2	61

[1] Data obtained from Figure 4.8.

[2] 1 mL CH_4 = 2.5 mg COD at 37°C and 1 atm. (See Appendix VI).

[3] See Appendix VI.

COD in the influent of the extracted H-coal wastewater feed solutions only account for ≈20% (Phase I) and ≈40% (Phase II) respectively. DIPE solvent may contribute a significant portion of the unknown COD. The anaerobic degradability and/or inhibition potential of DIPE is addressed later in this chapter.

Phase III (day 97-176)

On day 97 of semicontinuous draw and feed procedures, the cultures were introduced to pH 9 extracted H-coal wastewater feed, which reflected an increase in influent phenol of 520 to 833 mg/L. On day 106 the average effluent phenol concentration in the cultures maintained at 16.7 d HRT had accumulated to 72 mg/L (from 13 mg/L on day 97). Activated carbon was introduced to the cultures on day 109 by splitting each original culture (50 mL liquid volume) into two cultures (25 mL liquid volume each), one with 1,050 mg activated carbon/L and one without activated carbon. The feed volumes were reduced by 50% in order to maintain the same HRT.

Effluent phenol response of the cultures (16.7 d HRT) without activated carbon is shown in Figure 4.9. These cultures were monitored until day 131 when effluent phenol reached 350 mg/L and the cultures were abandoned. The concentration of phenol in the effluent did not follow the predicted washout concentration. The ability of these cultures to remove phenol was impaired as suggested by the phenol accumulation in the effluent, although removal clearly did not stop completely. The accumulation of some non-phenolic inhibitors is the most likely explanation for the deterioration in biological phenol removal as phenol concentrations

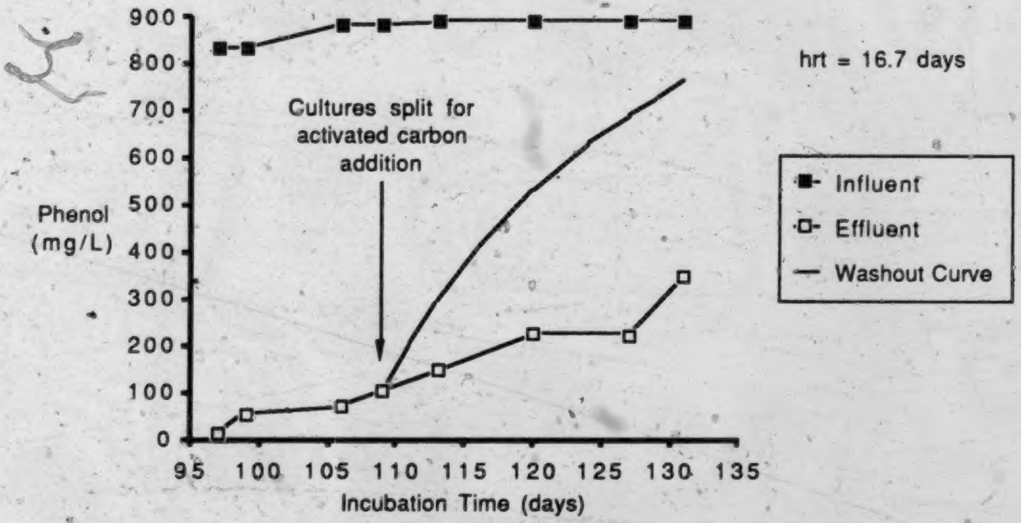

Figure 4.9 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater Without Activated Carbon - Phase III

were well below the tolerance level of anaerobic bacteria. The increase in effluent phenol concentration from day 127 to 131 parallels the expected washout concentration characteristics for this same time period (Figure 4.9), and cessation of biological phenol removal may have begun at this point.

Influent and effluent phenol concentrations in the cultures with activated carbon (16.7 d HRT) is shown in Figure 4.10. The presence of activated carbon improved the operating stability of the cultures for phenol removal. These cultures clearly performed better than the cultures without activated carbon (compare Figure 4.9 and 4.10). Activated carbon may have sequestered organic inhibitors from solution and allowed the anaerobic bacteria to operate under less stress than the cultures without activated carbon. The effluent phenol concentration increased immediately after the cultures were split and activated carbon was added on day 109 (Figure 4.10). Oxygen contamination during the setup procedure may have temporarily shocked the bacteria and impaired their ability to remove phenol from solution. Also, oxygen adsorbed to the activated carbon surface may have partially inhibited the bacteria when the activated carbon was first added to the cultures. The cultures with activated carbon had a quasi-stable operating period (day 133 to day 155).

Influent and effluent m/p-cresol concentrations in the same cultures is shown in Figure 4.11. Biological m/p-cresol removal was very unstable during this period and at best only provided 60% steady-state removal (day 133). Small increases in the influent m/p-cresol concentration (day 133) caused a sharp increase in effluent

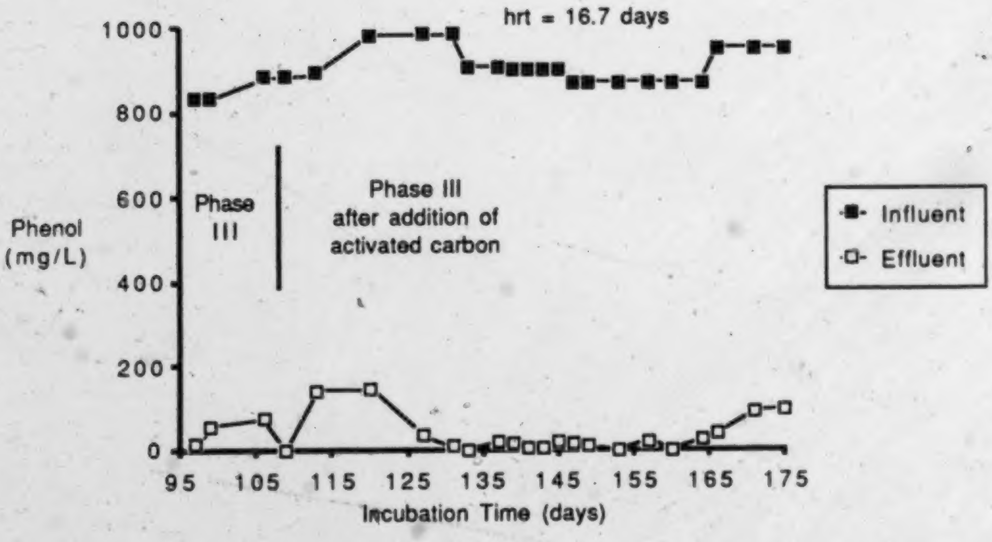

Figure 4.10 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater - Phase III

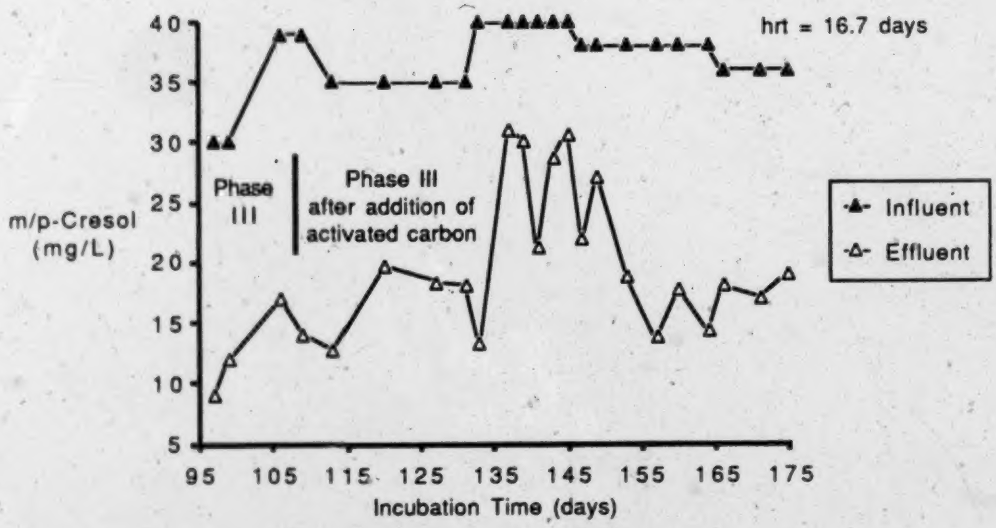

Figure 4.11 Semicontinuous Fermentation of m/p-Cresol in Extracted H-coal Wastewater - Phase III

m/p-cresol level. This period of increased m/p-cresol levels lasted from day 133 to 155.

Effluent phenol concentrations from day 72 to 167 for the cultures containing activated carbon (16.7 and 25 d HRT) were examined more closely in Figure 4.12. Between day 97 and 109, the effluent phenol accumulated to 72 mg/L until the addition of activated carbon (on day 109) for the 16.7 d HRT. After carbon addition phenol accumulated again to 146 mg/L on day 120. However, the effluent concentration rapidly dropped below 10 mg/L by day 131. Effluent phenol levels were low from day 133 to 155. Both sets of cultures had a quasi-stable period (day 133 to 155) where effluent levels were less than 20 mg/L. Neither sets of cultures exhibited features that indicated cessation of biological phenol removal (i.e. characteristics resembling washout concentrations shown in Figure 4.9). On day 175 the draw and feed procedures were suspended as phenol began accumulating in the effluent.

Methane production for both cultures is shown in Figure 4.13. Changes in the slope of the methane production rates were observed in both cultures from day 109 to 133 and day 133 to 155. The methane production rates are summarized in Table 4.10. The increased rates observed during day 133 to 155 agree with the period of improved phenol removal (quasi-stable operating period) for both sets of cultures shown in Figure 4.12. The methane production accounted for 66% (25 d HRT) and 57% (16.7 d HRT) of the influent COD during the period of improved phenol removal (day 133 to 155).

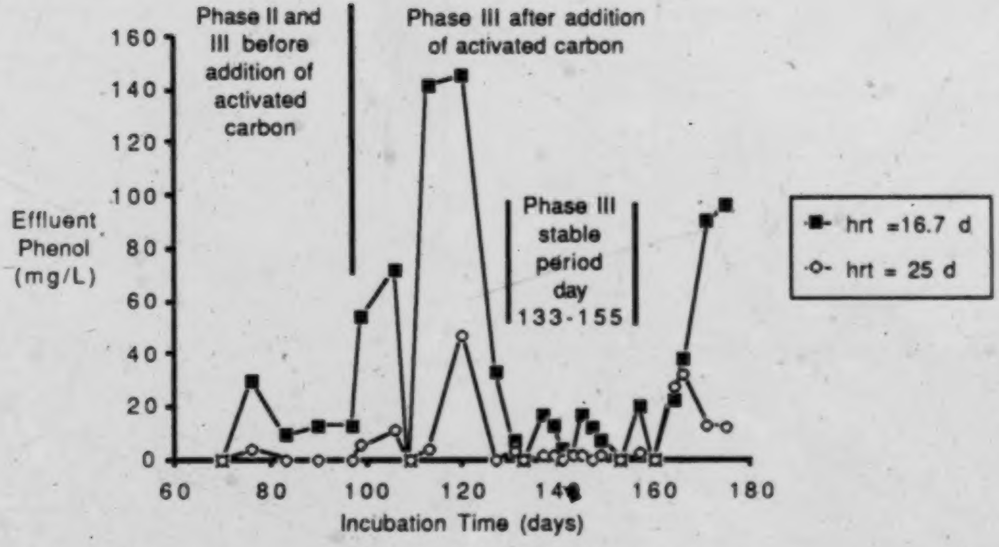

Figure 4.12 Effluent Phenol Concentrations in Cultures Containing Activated Carbon - Phase III

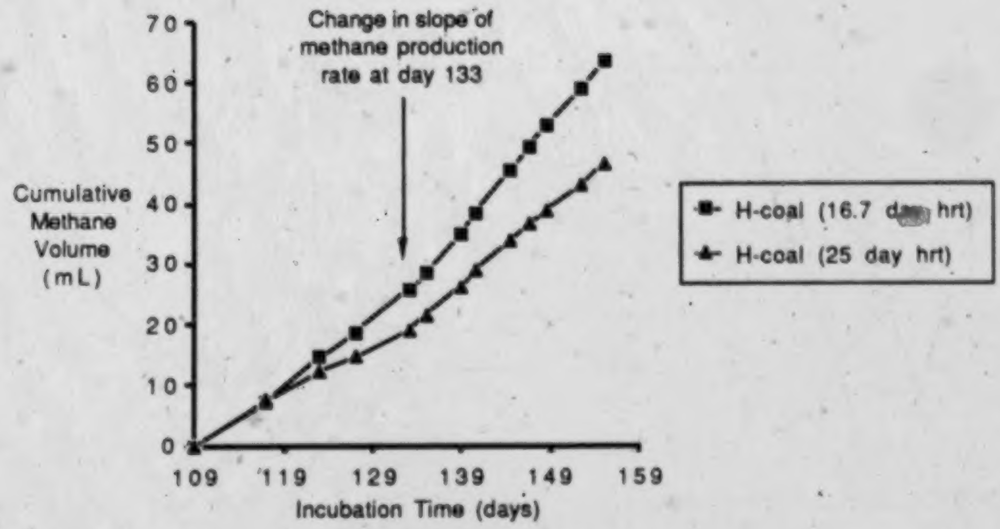

Figure 4.13 Methane Production during Semicontinuous Fermentation of Extracted H-coal Wastewater - Phase III

Table 4.10 Methane Generation Efficiency Based on COD Equivalence - Phases III and V

Culture	CH_4 Produced (mL/d)[1]	COD of CH_4 (mg/d)[2]	COD Fed (mg/d)[3]	COD Removed as CH_4 (%)
Phase III (25 day HRT):				
day 109-133	0.8	2.0	5.0	40
day 133-155	1.3	3.3	5.0	66
Phase III (16.7 day HRT):				
day 109-133	1.1	2.8	7.5	37
day 133-155	1.7	4.3	7.5	57
Phase V (12.5 day HRT):				
day 227-241	3.0	7.5	10.0	75

[1] Data obtained from Figure 4.13.
[2] 1 mL CH_4 = 2.5 mg COD at 37°C and 1 atm. (See Appendix VI).
[3] See Appendix VI.

It is interesting to note that the period of improved phenol removal and increased methane production (day 133 to 155, Table 4.10) coincides with the period of decreased m/p-cresol removal (Figure 4.11). This phenomenon suggests that the anaerobic bacteria may have had a shift in preference of the carbon source (i.e. from m/p-cresol to phenol) from day 133 to 155.

Phase IV (day 176-219)

The cultures were initially fed individually on demand during this period in an effort to improve their stability. Data for the worst case situation (a culture originally maintained at 16.7 d HRT) is presented to demonstrate the procedure used to nurse all the cultures back to stable operation. Using this procedure, it was possible to acclimate and operate all cultures at a HRT of 12.5 d.

Figure 4.14 demonstrates the influent and effluent phenol concentrations for the highest stressed (least active) culture. Phenol began accumulating in the culture on day 164. However, the phenol effluent concentrations did not follow the predicted washout concentrations (indicating a cessation of biological phenol removal in the absence of activated carbon). On day 175 the draw and feed procedure was stopped because it was evident the culture was not metabolizing phenol as quickly as it was being added. The culture was then analyzed from day 176 to 183. The phenol concentration declined from a high on day 183 (324 mg/L) to 167 mg/L on day 195 (when it was fed 77 mg/L). It was again fed 38 mg/L on day 201. Phenol accumulated to 220 mg/L on day 202, however, it declined steadily thereafter indicating that biological activity had improved.

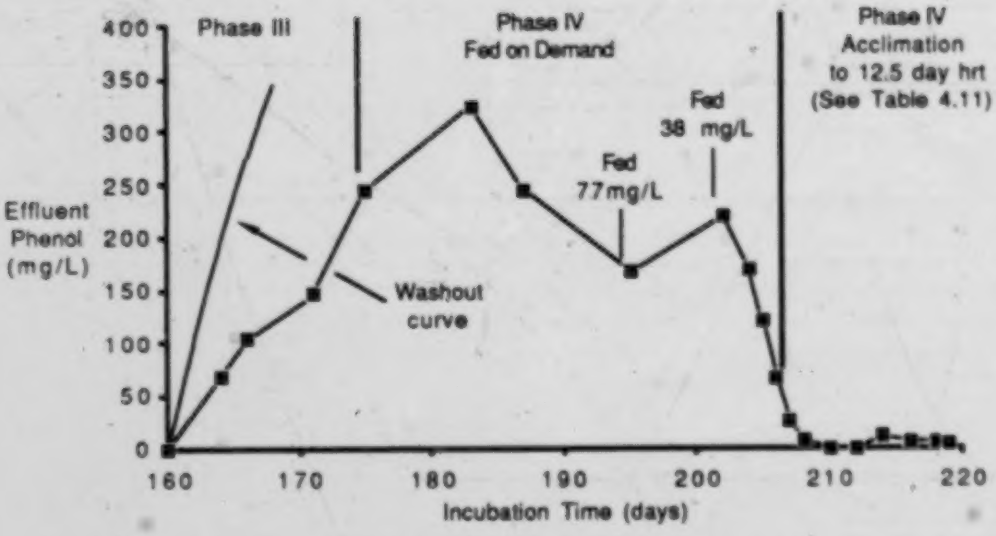

Figure 4.14 Effluent Phenol Concentrations of the Highest Stressed Culture Containing Activated Carbon - Phase IV

On day 207, daily draw and feeding of the culture was resumed according to the schedule in Table 4.11. Within 12 days (from day 207-219) the culture was processing phenol at an equivalent HRT of 12.5 d while maintaining effluent phenol levels below 11 mg/L. By day 219 all of the cultures (i.e. triplicate sets originally maintained at 16.7 and 25 d HRT) were processing phenol at an equivalent HRT of 12.5 d.

Phase V (day 219-250)

All cultures were maintained at the 12.5 d HRT for this period, and in general achieved excellent phenol removal (Figure 4.15). Effluent phenol concentrations were consistently maintained below 2 mg/L. The ability of the cultures to recover from the inhibition episode displayed earlier (Phase IV - Figure 4.14) demonstrates the resilience of the anaerobic bacteria, in the presence of activated carbon, to degrade phenol in a full strength phenolic wastewater.

Effluent concentrations of m/p-cresol during this period are shown in Figure 4.16. Approximately 30% m/p-cresol removal occurred during this time. These cultures had exhibited poor capability for m/p-cresol removal since day 20 (Figure 4.7). Even the addition of activated carbon on day 109 did not improve the removal. It is apparent that bacteria capable of degrading m/p-cresol did not become well established in the cultures under the conditions tested. Although m/p-cresol accounts for a minor portion of COD in the pH 9 wastewater (2%), the effluent concentrations experienced during Phase V (26-34 mg/L) are too high to allow the wastewater to be discharged. The presence of m/p-cresol in the effluent would require

Table 4.11 Draw and Feed Phenol Acclimation to 12.5 d HRT for Highest Stressed Culture - Phase IV

Incubation Time (days)	Aqueous Phenol (mg/L)	Daily Draw and Feed Volume (mL/day)[1]	Phenol Fed (mg/L)[2]	Equivalent HRT (days)[3]
207	25	1.0	42	25
208	7	"	"	"
209	<2	"	"	"
210	<2	1.25	52	20
211	<2	"	"	"
212	<2	"	"	"
213	<2	1.5	62	16.7
214	11	"	"	"
215	11	"	"	"
216	6	"	"	"
217	3	1.75	73	14.3
218	5	"	"	"
219	4	2.0	83	12.5

[1] Influent phenol concentration = 1,040 mg/L.

[2] Daily step increase of phenol in 25 mL cultures based on draw and feed volume.

[3] Hydraulic residence time based on draw and feed volume.

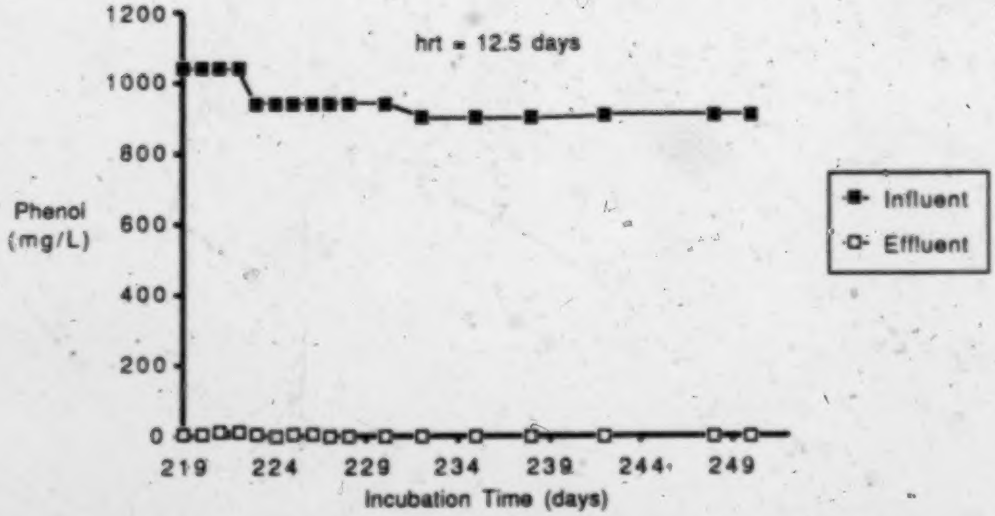

Figure 4.15 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater - Phase V

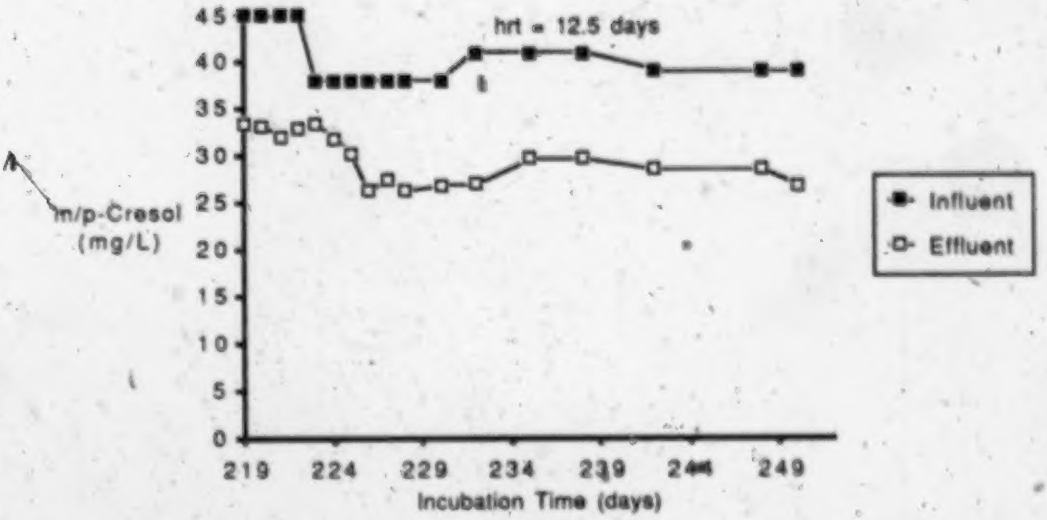

Figure 4.16 Semicontinuous Fermentation of m/p-Cresol in Extracted H-coal Wastewater - Phase V

further attention before the wastewater would be acceptable for discharge.

The expected and observed methane production during Phase V is shown in Figure 4.17. The difference in the expected and observed methane production is probably due to the GAC retention of COD and a small portion converted to biomass. The COD equivalence of the methane produced (Table 4.10) demonstrated that 75% of the influent COD was converted to methane.

A summary of the COD loading characteristics to all cultures during the 250 days of semicontinuous (draw and feed) operation is provided in Table 4.12. The cultures received COD loading rates of 0.1 to 0.4 $\frac{mg}{mL \cdot d}$. An important feature of Table 4.12 is the COD converted to methane theoretically accounts for only 57 to 78% of the influent COD loading. The portion not converted is substantial. For example, the Phase V influent COD (5,000 mg/L) was only 75% converted to biological methane. The COD in the effluent would still require further treatment to produce an effluent acceptable for discharge.

4.5 Semicontinuous Fermentation Studies with DIPE

DIPE is soluble in water (8,940 mg/L at 25°C - see Appendix II) and its presence in the extracted H-coal wastewater may have been responsible for some of the inhibition episodes experienced in the fermentation study in section 4.4. Or, because the anaerobic biodegradation potential is unknown, the presence of DIPE may have

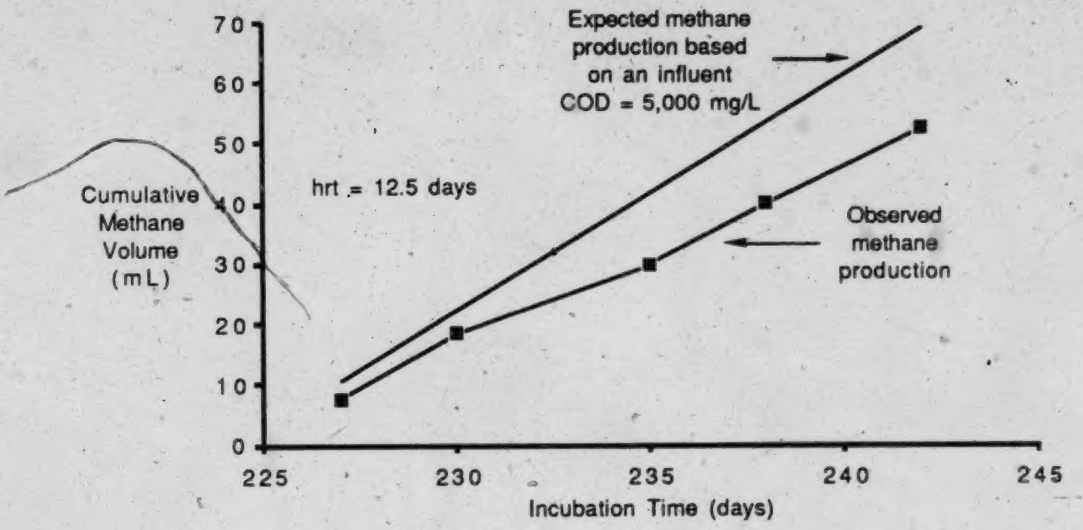

Figure 4.17 Methane Production during Semicontinuous Fermentation of Extracted H-coal Wastewater - Phase V

Table 4.12 Semicontinuous COD Loading Characteristics of Extracted H-coal Wastewater

Culture	Liquid Volume (mL)	COD Fed (mg/d)	COD Loading Rate ($\frac{mg}{mL \cdot d}$)	COD Removed as CH_4 (%)
Phase I (day 1-73):				
25 day HRT	50	4.9	0.10	78
16.7 day HRT	50	7.4	0.15	68
Phase II (day 73-97):				
25 day HRT	50	7.4	0.15	72
16.7 day HRT	50	11.2	0.22	61
Phase III (day 97-176) (after activated carbon addition)[1]:				
25 day HRT	25	5.0	0.20	66
16.7 day HRT	25	7.5	0.30	57
Phase V (day 219-250):				
12.5 day HRT	25	10.0	0.40	75

[1] Data from the stable period (day 133-155) was used.

added excess substrate loading to the anaerobic process. The purpose of this study was to:

i) determine if water soluble DIPE was biodegradable or inhibitory to anaerobic bacteria during phenol degradation, and

ii) examine the extent to which the soluble DIPE influenced the organic composition of the H-coal wastewater.

4.5.1 Procedures

A semicontinuous draw and feed experiment was performed to assess the biodegradability or inhibition potential of DIPE during anaerobic phenol degradation. All conditions tested were performed with triplicate cultures and incubated at 37°C. Initially, 45 mL of domestic anaerobic sludge and 5 mL of a growth medium were added to 119 mL flushed serum bottles and incubated 24 h prior to being fed.

One feed solution contained tap water supplemented with phenol. The other feed solution contained DIPE-extracted tap water supplemented with the same level of phenol as the tap water feed solution. The batch extraction procedure was performed by contacting the tap water with DIPE solvent three times at a measured tap water to solvent volume of 10:1 (the same procedure for extraction of the H-coal wastewater).

The feed solutions were prepared by anaerobically dispensing 90 mL of the phenol-supplemented tap waters along with 10 mL of the nutrient growth medium into 119 mL flushed serum bottles, which were then capped and sealed. A 1 mL portion of sulfide solution (Table 3.1) was added by needle and syringe to ensure a reduced feed.

A blank feed solution using tap water (without phenol) was set up in similar fashion. A daily draw and feed procedure was started with the cultures to give them a HRT of 16.7 days (3 mL/day for the 50 mL cultures). The cultures were maintained for 72 days.

DIPE influent and effluent concentrations were monitored periodically during Phase V of the semicontinuous anaerobic fermentation study with the extracted H-coal wastewater. The effluent was collected from day 229 to 240 and stored in a sealed container at 4°C. A 100 mL portion of the collected wastewater was allowed to cool to room temperature and then air sparged at 250 mL/min (by placing a sparging stone in the wastewater) and the removal of DIPE was monitored.

4.5.2 Results and Discussion

Response of anaerobic cultures to phenol supplemented tap water (Figure 4.18) and phenol supplemented DIPE-extracted tap water (Figure 4.19) demonstrates that DIPE did not impair or inhibit the cultures from removing phenol. It is, therefore likely that the inhibition episodes observed during fermentation of the DIPE-extracted H-coal wastewater were due to compounds (other than phenol and DIPE) not completely removed during extraction.

Methane production from both of these cultures (plotted in Figure 4.20) show that DIPE was not biologically converted to methane under these anaerobic conditions.

Effluent DIPE concentrations observed during Phase V of the H-coal semicontinuous fermentation study are shown in Table 4.13. An influent concentration of 725 mg/L (2,040 mg COD/L) was measured

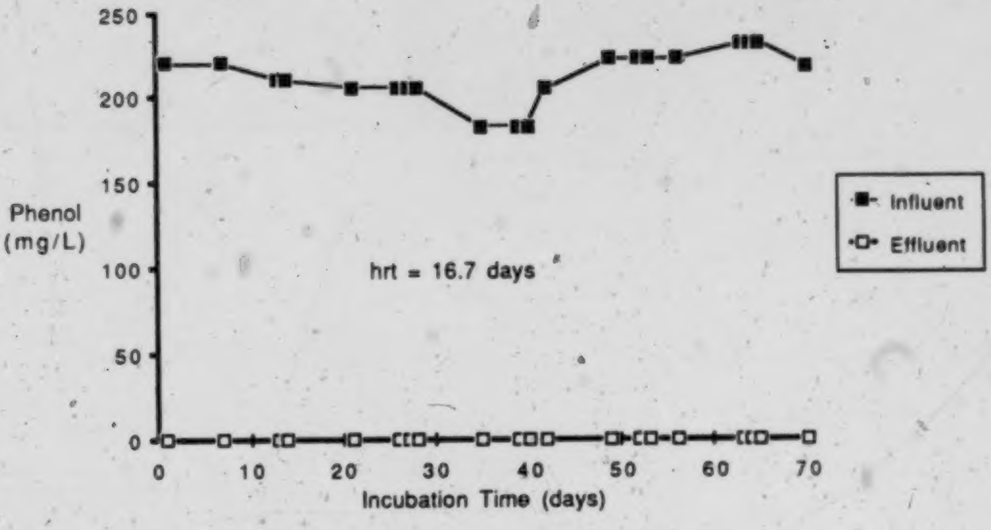

Figure 4.18 Semicontinuous Fermentation of Phenol in Tap Water

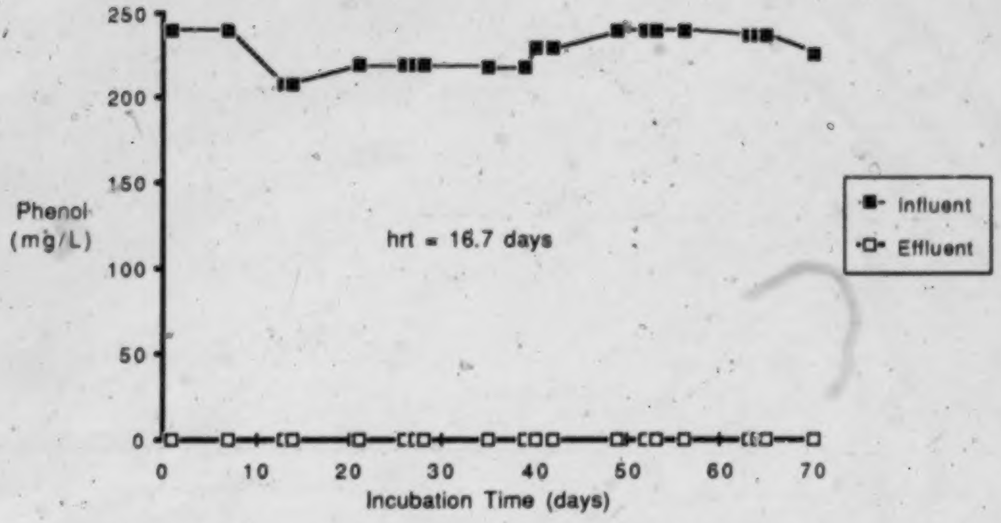

Figure 4.19 Semicontinuous Fermentation of Phenol in DIPE Extracted Tap Water

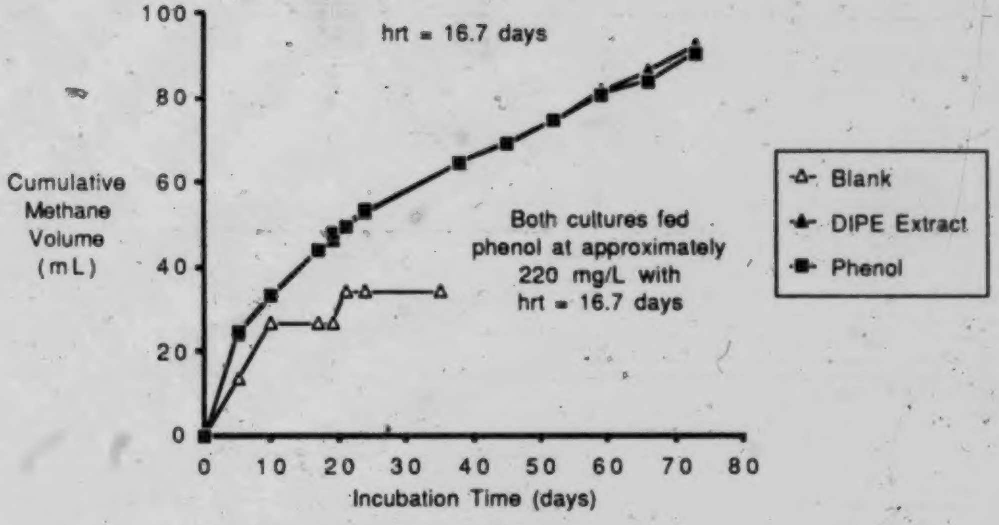

Figure 4.20 Methane Production During Semicontinuous Fermentation of Phenol in Tap Water and DIPE Extracted Tap Water

Table 4.13 Effluent DIPE Concentrations Measured During Semicontinuous Anaerobic Fermentation of Extracted H-coal Wastewater - Phase V

Incubation Time (days)	DIPE (mg/L)[a]
225[b]	455-496
226[c]	325-335
232[b]	447-490
235[d]	340-360
242[b]	447-530
245[d]	340-375

Note: Influent DIPE = 725 mg/L (from day 239-250).

[a] Concentration range of effluent DIPE in 6 cultures maintained at 12.5 day HRT.

[b] Prior to flushing of the serum bottle headspace gas (see section 3.2.4).

[c] One day after flushing the bottles (see section 3.2.4).

[d] Three days after flushing the bottles (see section 3.2.4).

during this period. Two characteristic ranges of effluent DIPE were observed: 450-530 mg/L prior to flushing the serum bottle headspace for volumetric methane measurements, and 325-375 mg/L after the flushing procedure. The serum bottles were routinely flushed with an O_2-free 30% CO_2 in N_2 to allow the accurate measurement of methane gas over a long period of time (see section 3.2.4). Neither concentration range of DIPE appeared to affect the performance of the anaerobic cultures. It is likely that at this stage of the study (i.e. after 220 days draw and feed) the anaerobic cultures were acclimated to the presence of DIPE.

The influent DIPE accounted for 41% of the influent COD (calculated at 5,000 mg/L - see Appendix VI), while the two levels of effluent DIPE accounted for 25-30% (450-530 mg/L) and 18-21% (325-375 mg/L) of the influent COD. Methane production during this phase accounted for 75% of the influent COD (Table 4.12). The presence of DIPE contributes a substantial portion of the effluent COD and requires further attention before the wastewater would be acceptable for discharge.

Results of air stripping the anaerobic H-coal effluent are shown in Figure 4.21. The procedure was able to remove all of the DIPE from the effluent. The wastewater requires solvent removal (either prior to or after anaerobic treatment). Aerobic biological treatment studies of solvent extracted coal conversion wastewaters had similar findings (see section 2.2.2.3).

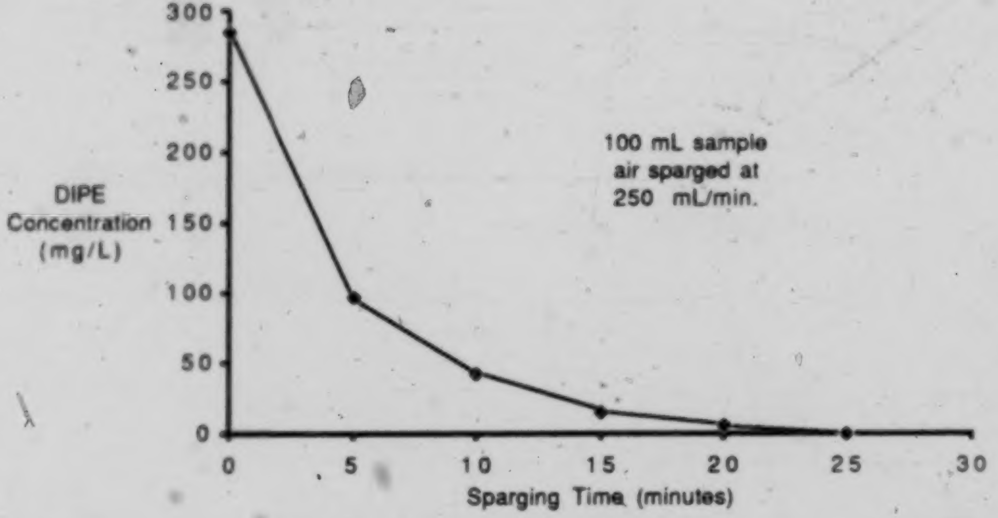

Figure 4.21 Results of Air Stripping DIPE from Anaerobically-Treated DIPE-Extracted H-coal Wastewater

4.6 Anaerobic Toxicity Assay with Aniline Compounds

Two of the non-phenolic compounds tentatively identified and quantitated during GC/MS analysis of the spent DIPE solvent fraction were aniline and an isomer of methylaniline (see Table 4.5). Both compounds are slightly soluble in water and their extraction efficiency was unknown. It was decided to test the inhibition potential of aniline and N-methylaniline with respect to anaerobic phenol degradation in batch toxicity assays.

4.6.1 Procedures

The batch assays were performed in 58 mL serum bottles that were anaerobically flushed prior to use. All conditions tested were performed with triplicate cultures and incubated at 37°C. Constituents added to the serum bottles are shown in Table 4.14. The total liquid volume of all cultures was 12.5 mL. A set of blank cultures (i.e. without phenol or a test compound) and a set of cultures that received phenol (without a test compound) were set up. The concentration range of the test compounds was chosen to bracket their suspected level present in the original un-extracted H-coal wastewater (see Table 4.5). All cultures (except the blank) were initially supplemented with approximately 640 mg/L synthetic phenol.

4.6.2 Results and Discussion

Response of anaerobic phenol-degrading cultures to different aniline concentrations is shown in Figure 4.22, and response to different N-methylaniline concentrations is shown in Figure 4.23.

Table 4.14 Serum Bottle Constituents for Anaerobic Toxicity Assay of Aniline Compounds.

Constituents	Volume	Concentration[1]
Nutrient growth medium	4.0 mL	-
Municipal digestor sludge	4.8 mL	-
Phenol acclimated sludge	3.2 mL	-
Phenol stock (16,000 mg/L)	0.5 mL	640 mg/L
Test Compounds:		
1. Aniline (4,924 mg/L)	3 µL	1 mg/L
2. "	25 µL	10 mg/L
3. "	75 µL	29 mg/L
4. "	250 µL	97 mg/L
1. N-Methylaniline (1,464 mg/L)	8 µL	1 mg/L
2. "	25 µL	3 mg/L
3. "	85 µL	10 mg/L
4. "	250 µL	29 mg/L

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[1] Serum bottle concentration.

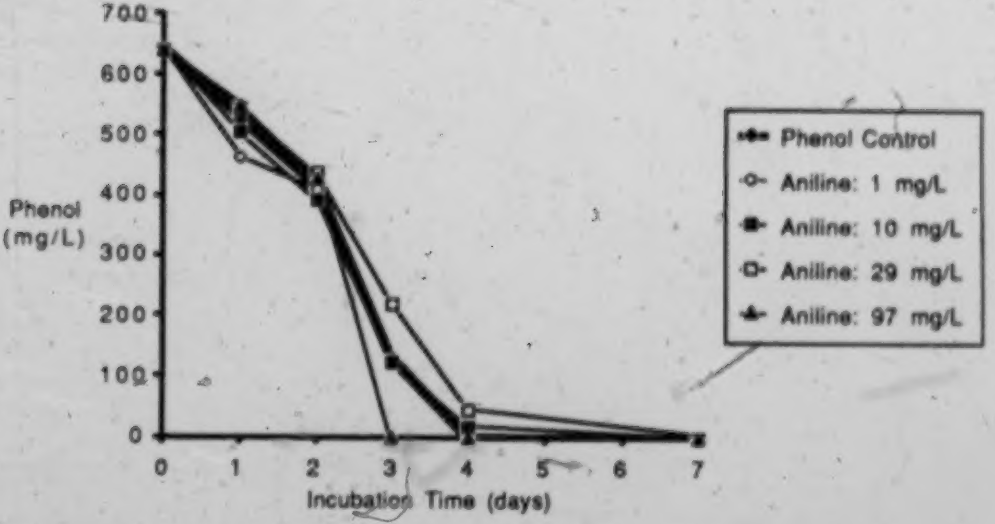


Figure 4.22 Batch Fermentation of Phenol in the Presence of Aniline

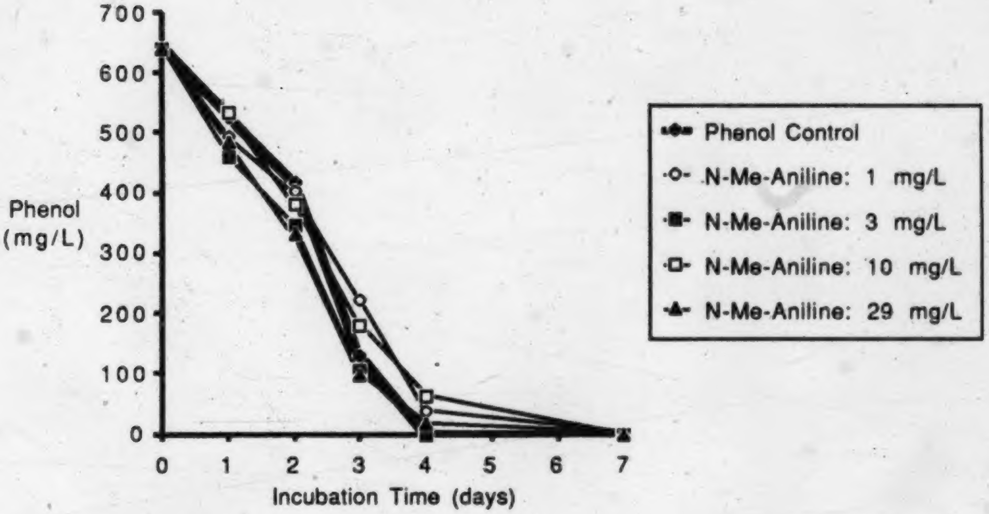


Figure 4.23 Batch Fermentation of Phenol in the Presence of N-Methyl Aniline

Neither compound appeared to impair or inhibit the ability of anaerobic bacteria to remove phenol when compared with the phenol control culture. The majority of phenol was rapidly removed within 4 days in all of the cultures. This was probably due to the high seed concentration of anaerobic bacteria used (Table 4.14).

The methane produced from all the cultures at day 7 is shown in Table 4.15. The initial phenol concentration of 640 mg/L is equivalent to 19 mg of COD per 12.5 mL liquid volume (1 mg/L phenol = 2.38 mg/L COD; see Appendix II). The average methane produced (in excess of the blank culture) from all the cultures in Table 4.15 was 11.9 - 6.5 = 5.4 mL. The COD value of this methane is only 13.5 mg (1 mL CH_4 = 2.5 mg COD at 37°C and 1 atm.; see Appendix VI). Although the methane produced did not compare with the available influent phenol (in terms of COD), it is evident the methane producing bacteria were active. The incubation time (7 days) may have been too short to allow complete conversion of all the biodegradable COD (19 mg) to methane.

Aniline (and N-methylaniline) did not inhibit anaerobic cultures at the highest concentration tested - 97 mg/L (29 mg/L), neither were they biodegraded during the 7 day test period. Blum et al. (1986) found that aniline, at a concentration of 100 mg/L, was not degraded by anaerobic consortia after 28 weeks incubation.

Solvent extraction and removal of anilines from the H-coal wastewater may not be a prerequisite to anaerobic treatment, however it is preferred (to produce an effluent of acceptable discharge quality). Concentrations of anilines in the pH 9 extracted

Table 4.15 Methane Production During Anaerobic Toxicity Assay of Aniline Compounds

	Test Condition	mL of CH_4 on Day 7
	Blank	6.5
	Phenol control	12.0
1.	Aniline (1 mg/L)	11.9
2.	" (10 mg/L)	11.9
3.	" (29 mg/L)	11.6
4.	" (97 mg/L)	12.4
1.	N-Methylaniline (1 mg/L)	11.6
2.	" (3 mg/L)	12.5
3.	" (10 mg/L)	11.7
4.	" (29 mg/L)	11.9

Note: Methane volumes are calculated at 37°C and 1 atm. All cultures (except Blank) contained 640 mg phenol/L at day 0.

wastewater were not responsible for the instability of the semicontinuous phenol-degrading cultures.

4.7 Summary

Adjusting the pH of H-coal wastewater and extracting with DIPE is an effective method for reducing the biodegradable phenolic concentration to a maximum level amenable to anaerobic bacteria. The weak acidic nature of phenol and substituted phenols allows for their selective removal by solvent extraction. Batch extraction trials at pH values of 7 to 11.5 with DIPE demonstrated that COD concentrations could be substantially reduced.

Phenolic, m/p-cresol, and o-cresol were primarily responsible for the buffer characteristics exerted by H-coal wastewater in the 9.6-10.2 pH range.

GC/MS analysis of the spent DIPE fraction identified a host of organic compounds that, in part, accounted for the background matrix of organic pollutants removed from the H-coal wastewater.

Anaerobic bacteria were able to actively degrade phenol in solvent extracted H-coal wastewater under batch and semicontinuous loading conditions. Solvent extraction removed unknown non-phenolic compounds that were previously shown to be inhibitory to anaerobic bacteria in batch cultures. However, the phenol-degrading cultures receiving H-coal wastewater on a semicontinuous basis exhibited instability. The same cultures displayed poor capacity for m/p-cresol removal and did not degrade o-cresol.

The immediate addition of activated carbon to semicontinuous phenol-degrading cultures relieved the short term stress, however the cultures eventually became inhibited. The inhibition was only temporary as the cultures were nursed back to efficient operation by interrupting and controlling the phenol feed.

Anaerobic activated carbon treatment of solvent extracted H-coal wastewater did not produce an effluent with acceptable discharge quality. Further treatment would be necessary to remove residual COD present in the effluent.

DIPE was neither inhibitory to anaerobic bacteria nor was it biodegraded to methane. The presence of DIPE did represent an excess COD loading to the wastewater and its removal is preferred. Air stripping of anaerobically treated H-coal wastewater completely removed soluble DIPE.

Aniline and N-methylaniline were two major contaminants tentatively identified in un-extracted H-coal wastewater by GC/MS analysis. Neither compound inhibited anaerobic bacteria from degrading phenol at their estimated concentration in the un-extracted wastewater.

5.0 THE IMPORTANCE OF A BIOLOGICAL SUPPORT DURING ANAEROBIC PHENOL DEGRADATION

Activated carbon was shown to be beneficial to anaerobic treatment of solvent extracted H-coal liquefaction wastewater. The addition of activated carbon to stressed phenol-degrading cultures improved their ability to remove phenol from solution. Similarly stressed cultures which did not receive activated carbon continued to accumulate phenol. The reported mechanisms by which activated carbon enhanced the anaerobic culture performance were discussed in section 2.3.3. This chapter will further assess the role of activated carbon and non-adsorptive ion exchange resins as biological supports and attempt to identify features that enhance anaerobic phenol degradation.

Three support materials studied were:

i) Filtrasorb® 300 activated carbon (average size 420-850 µm) (Calgon Carbon Corp.),

ii) Ambergard™ XE-352 anion exchange resin (average size 300-850 µm) (Rohm and Haas Co.), and

iii) Dowex™ MSC-1 cation exchange resin (average size 300-850 µm) (Dow Chemical Co.).

5.1 Support Surface Characterization

The characterization methods investigated both physical and chemical features of activated carbon and physical features of the synthetic ion exchange resins. The synthesis of ion exchange resins allow for the controlled preparation of its chemical structure.

Information on surface chemistry of the resins was supplied by the manufacturer.

5.1.1 Activated Carbon

Activated carbon is made by a pyrolytic process that results in a microcrystalline structure composed of parallel graphite planes stacked in unordered fashion (McGuire and Suffet, 1978). Filtrasorb® 300 (F300) activated carbon is derived from a bituminous coal and activated under rigidly controlled conditions with high temperature steam (Calgon Corp., 1985). The activation procedure creates a well developed pore structure with extensive internal surface. Much of the surface functionality of activated carbon originates during activation.

5.1.1.1 Surface Chemistry

The carbon-oxygen complexes are the most predominant surface functional group influencing the physicochemical properties of carbon materials (Kinoshita, 1988). The surface oxygen complexes are produced by gas phase oxidation with various oxidizing agents (e.g. O_2, N_2O, CO_2, H_2O, and high temperature steam). Several different types of surface oxide groups have been proposed (Kinoshita, 1988):

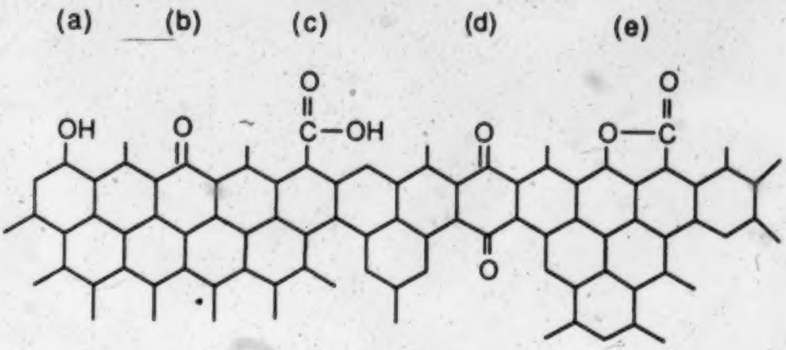
i) phenolic hydroxyl,
ii) carbonyl,
iii) carboxyl,
iv) quinone (dual carbonyl), and
v) lactone (cyclic ester).

Figure 5.1 depicts the graphitic structure of a layer (or plane) of activated carbon with the proposed oxygenated functional groups situated on the edge of the plane. Snoeyink and Weber (1967) stated that the sides (or edges) of the planar surfaces would contain the attached functional groups due to the greater tendency of these positions to participate in electron sharing reactions which characterize chemisorption of the oxidizing gases to the surface.

Two approaches were used to try to quantify oxygen-associated surface functionality. Elemental analysis, neutron activation analysis, and x-ray photoelectron spectroscopy were used to measure the percent (by mass) oxygen concentration of activated carbon. Diffuse reflectance IR spectroscopy, direct transmission IR spectroscopy, photoacoustic IR spectroscopy, and x-ray photoelectron spectroscopy were used to identify individual oxygen fuctional groups on the surface.

Elemental analysis was performed in triplicate and yielded mass oxygen concentrations of 1.4, 1.3, and 1.4%. The experimental error for the determination was ± 0.3%.

Neutron activation analysis is considered a more accurate technique for mass oxygen measurement. However, a direct oxygen mass measurement was impossible due to the high aluminum content in the activated carbon (estimated at ≈2% by mass). High Compton background produced by ^{28}Al interfered


(a)
(b)
(c)
(d)
(e)
OH
O
O
C—OH
O
O
O—C
O

(a) Phenolic hydroxyl

(b) Carbonyl

(c) Carboxyl

(d) Quinone

(e) Lactone

Figure 5.1 Oxygenated Surface Functional Groups of Activated Carbon
(Adapted from Kinoshita, 1988)

with the oxygen determination. The Compton background phenomena occurred from the following reaction:

$$ {}^{27}_{13}\mathrm{Al} + {}^{1}_{0}\mathrm{n} \text{ ===> } {}^{28}_{13}\mathrm{Al} + ¥. \qquad \text{(Eqn. 5-1)} $$

The high energy (1,779 keV) of the ¥ particle emitted by ^{28}Al from the reaction in equation (5-1) produced Compton background scattering. This phenomena occurs from the interaction of ¥ particles with electrons far away from the attraction of a nucleus. When a collision takes place between a ¥ particle and an electron, the scattered ¥ particle has less energy (as some of the energy is lost to the electron). This scattering can occur again and again, and produces a characteristic broad background intensity signal related to the different reduced energies of scattered ¥ particles. The Compton effect obstructed the measurement of 197 keV ¥ particles emitted by ^{19}O.

Photoacoustic IR spectroscopy (PAS) and Diffuse Reflectance IR Spectroscopy (DRS) did not reveal any information of surface functional groups on activated carbon. During PAS analysis the activated carbon sample strongly absorbed infrared radiation and conducted heat. The acoustic signal was saturated as a result, and no absorption bands could be identified in the 4,000 to 400 cm^{-1} wavenumber range. Similarly, DRS analysis of activated carbon produced an infrared absorption spectra with no identifiable bands or peaks in the 4,000 to 400 cm^{-1} wavenumber range. The reason for the flat signal was unknown.

Direct transmission IR spectroscopy (DTS) revealed information on the surface chemistry of activated carbon. Figure 5.2 shows the infrared radiation absorption spectra of a KBr pellet containing F300 activated carbon. The increasing absorbance with increasing wavenumber (decreasing radiation wavelength) is attributed to scattering of infrared radiation from solid activated carbon particles and shows up as increased absorption in the absorption spectra. The scattering effect is more pronounced at the longer wavenumbers. Three absorption intensity peaks were observed (3420 cm^{-1}, 1630 cm^{-1}, and a broad peak in the 1300-900 cm^{-1} range).

The spectra in Figure 5.2 did not take into account moisture adsorption that can occur during preparation of the pelletized KBr disc (Zawadzki, 1978). Figure 5.3 shows the absorption spectra of adsorbed moisture in the KBr pellet in the absence of activated carbon. The absorption baseline signal was characteristically flat. Two absorption intensity peaks were observed (3440 cm^{-1} and 1625 cm^{-1}). O'Reilly and Mosher (1983) reported that moisture bands occur in the 3600-3400 cm^{-1} and 1640 cm^{-1} region associated with OH absorption, and Hergert (1971) also stated that a band in the 1625 cm^{-1} range is due to moisture.

Figure 5.4 shows the absorption spectra of F300 activated carbon with moisture subtracted. The absorbance intensity scale was enlarged to allow identification of absorbance

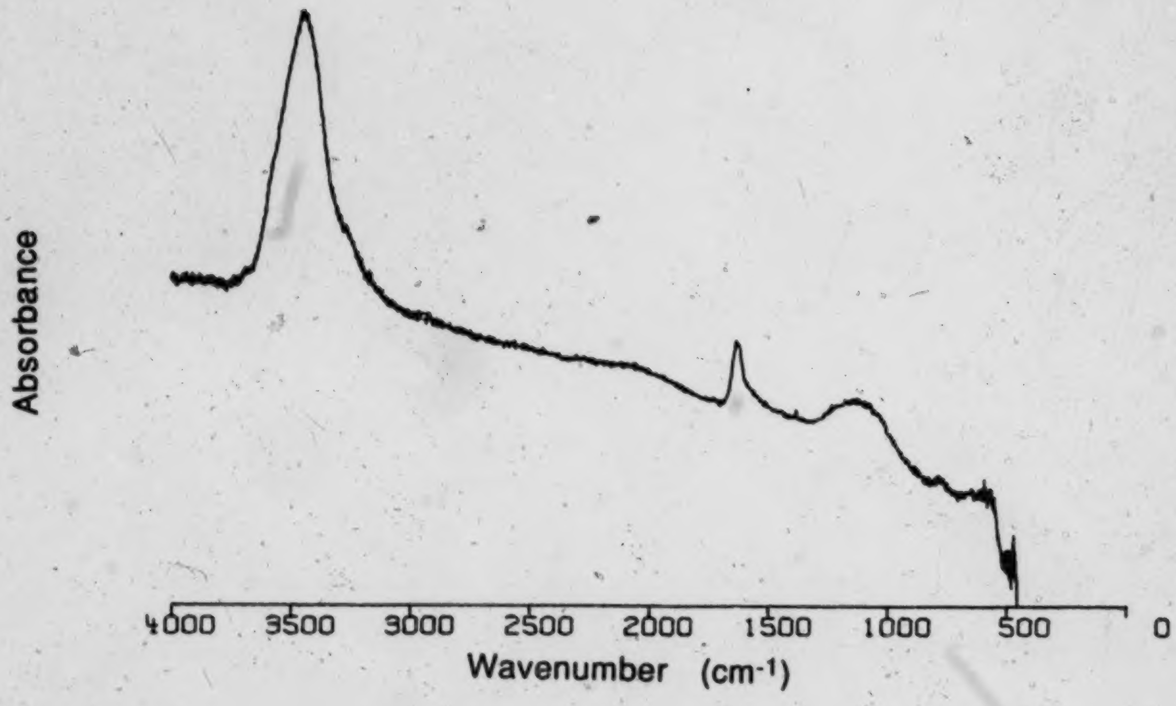


Figure 5.2 Direct Transmission FTIR Absorption Spectra of F300 Activated Carbon Plus KBr (Vertical scale: 1 cm = 0.011 absorbance units)

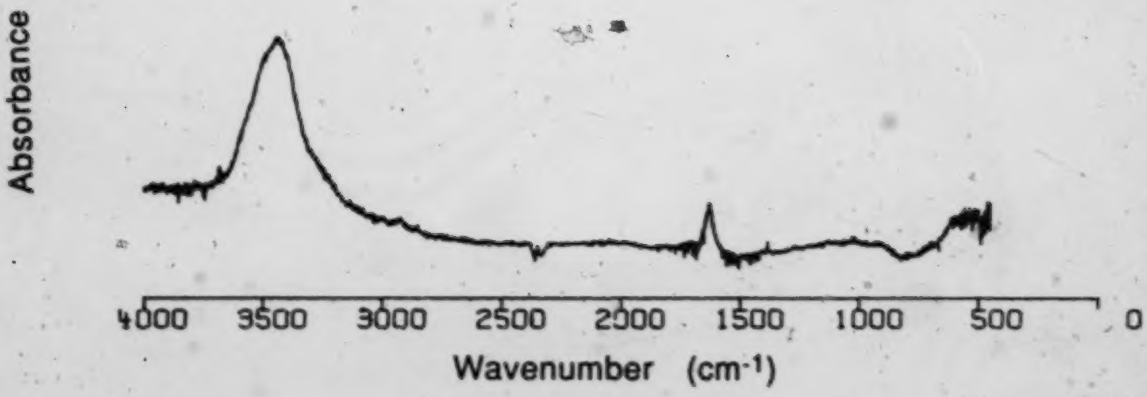


Figure 5.3 Direct Transmission FTIR Absorption Spectra of KBr (Moisture)
(Vertical scale: 1 cm = 0.011 absorbance units)

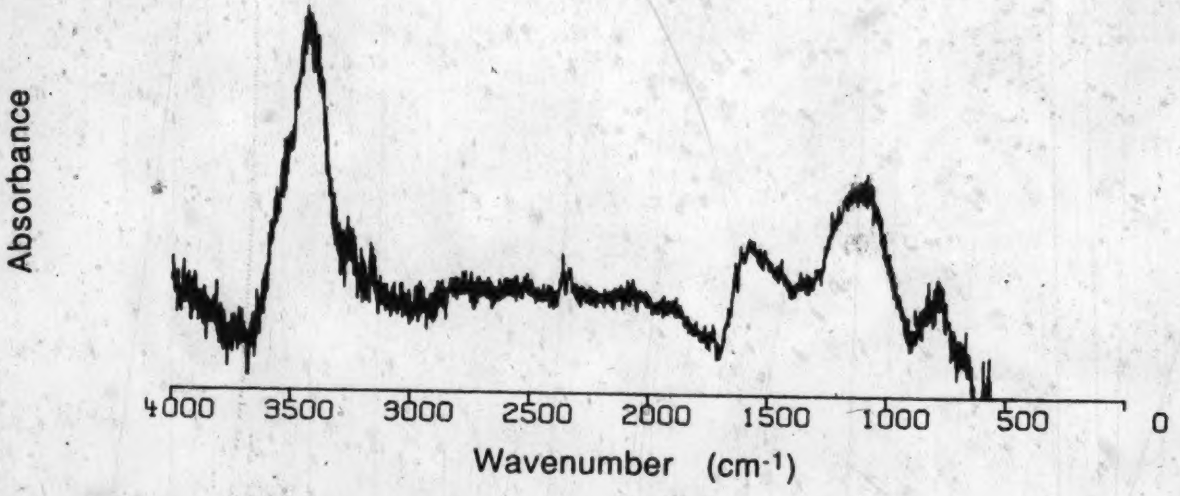


Figure 5.4 Direct Transmission FTIR Absorption Spectra of F300 Activated Carbon with Moisture Subtracted (Vertical scale: 1 cm = 0.034 absorbance units)

peaks. Four peaks were observed. A sharp peak at 3440 cm^{-1}, a broad peak in the 1700-1400 cm^{-1} range, a broad peak in the 1300-900 cm^{-1} range, and a broad peak observed between 900-600 cm^{-1} wavenumbers. The region below 1400 cm^{-1} is typically referred to as the fingerprint region. The absorption intensity signal in this region represents a complex mixture of bending and stretching vibrations.

Interpretation of absorption spectra of solids is different compared to liquids because the absorption signals are not as sharp from solids. Also, bands that occur close together tend to overlap. Hence, the absorption signal for the same type of chemical bond may occur at a slightly different wavenumber for a solid and liquid. The strong peak at 3440 cm^{-1} is attributed to phenolic hydroxyl (-OH) stretching vibrations. Zawadzki (1978) reported that oxidation of cellulose exhibited an -OH stretching band at 3450 cm^{-1}. Painter et al. (1981) associated the 3450 cm^{-1} band with -OH stretching from infrared spectra of coal.

The broad peak observed in the 1700-1400 cm^{-1} range was not assigned. Ishizaki and Marti (1981) studied the infrared spectra of Filtrasorb® 200 activated carbon. They reported the following band assignments near this region: 1760-1710 cm^{-1} (C=O stretching vibrations of lactonic and carboxylic groups), 1670-1500 cm^{-1} (C=O stretching vibrations of carboxyl), and 1480-1340 cm^{-1} (-OH bending vibrations). Ishizaki (1984) and Kinoshita (1988) assigned 1735-1725 cm^{-1} band to acidic carbonyl groups during spectral analysis of activated carbon.

The broad bands observed below 1300 cm^{-1} wavenumbers were not distinct enough to be assigned. Kinoshita (1988) summarized band assignments from spectral analysis of activated carbon in the 1400-1000 cm^{-1} range: 1480-1340 cm^{-1} (phenolic associated C-OH), 1260 cm^{-1} (C-O-C lactone vibration), 1215 cm^{-1} (C-O absorption), and 1180-1000 cm^{-1} (C-O stretch and vibration).

The presence of carbon-oxygen groups on the surface of F300 activated carbon appears likely. Other than phenolic hydroxyl functionality, results of direct transmission IR spectroscopy did not distinguish different types. The broad bands in the 1700-1400 and 1300-900 cm^{-1} range indicate the possible presence C-O functionality on the surface of activated carbon. However, the 1300-900 cm^{-1} band could be associated with phenolic hydroxyl (i.e. C-OH).

Analysis of the F300 activated carbon by x-ray photoelectron spectroscopy (XPS) provided similar information. Widescan XPS spectra for the sample and high resolution narrow scan XPS spectra on oxygen (1s) and carbon (1s) peaks are located in Appendix VII. Table 5.1 summarizes the quantitative surface composition of the sample determined from the widescan XPS spectra. The estimated error of precision is 10-15% of reported values in Table 5.1.

The major elements observed were carbon and oxygen. The highly graphitic nature of activated carbon accounts for the significant carbon content (92.2%). Hydroxyl (OH) was evident on the surface. There did not appear to be any evidence for the

Table 5.1 Surface Composition of F300 Activated Carbon Based on XPS Survey

Element	Surface Composition (%)[1]
Fe	0.2
O	6.1
C	92.2
S	0.2
Si	0.6
Al	0.7

[1] Averaged over a depth of 40 μm.

Note: Error of surface composition is 10-15% of reported values.

presence of carboxyl (COOH) or carbonyl (C=O) species in the sample. However, high resolution scans for oxygen (1s) spectra (Figure VII.2) suggest there is more than one oxygen species present. These oxygen complexes could be associated with carbon or other impurities listed in Table 5.1.

Elemental analysis and XPS provided evidence of the presence of oxygen in F300 activated carbon. DTS and XPS indicated that the only identifiable oxygen complex present (of the proposed complexes in Figure 5.1) was phenolic hydroxyl (OH). However, both techniques indicated the presence of other oxygen complexes of less significance.

5.1.1.2 **Surface Morphology**

The microporous surface area of F300 activated carbon was determined by BET isotherm analysis of nitrogen gas adsorption. The results are summarized in Table 5.2. Most of the internal surface area of activated carbons is associated with micropores. A total surface area of 570.7 m^2/g was calculated for the F300 activated carbon. The manufacturer cites an available surface area of 950-1,050 m^2/g based on similar BET analysis (Calgon Corp., 1985). Essentially none of the micropore volume and associated surface area would be available for bacteria with a size diameter ≥ 0.3-2 μm (average size diameter of single-celled bacteria; Nester et al., 1983). Micropores would provide adsorption cites for organic compounds.

Table 5.2 Micropore and Surface Area Distribution of F300 Activated Carbon Based on BET Method

Pore Diameter ($*10^{-2}$ µm)	Pore Volume (mL/g)	Pore Surface Area (m^2/g)
>6	0.0740	1.722
6 - 4	0.0103	0.818
4 - 2	0.0198	2.831
2 - 1	0.0193	5.275
1 - 0.8	0.0062	2.781
0.8 - 0.6	0.0083	4.896
0.6 - 0.4	0.0070	6.183
0.4 - 0.2	0.0300	41.155
0.2 - 0.1	0.0256	57.981
<0.1	-	447.172
Total =	0.2005	570.714

Lee and Snoeyink (1980) measured the surface area and pore volume of granular activated carbons (GAC) from several manufacturers. GAC derived from bituminous coal, lignite coal, petroleum-based coke, and wood-based coal were analysed by BET nitrogen gas adsorption isotherms. Total surface areas of 861-1,422 m^2/g, and pore volumes of 0.612-1.865 mL/g were reported based on pore diameters $\geq 10^{-3}$ µm. The total surface area and pore volumes reported for these activated carbons are substantially different than the measured value of F300 (570.7 m^2/g). The reproducibility and accuracy of the BET method for surface area analysis is unknown. Montgomery (1985) reported that the effective surface area (i.e. area available for adsorbates) for GAC has an upper limit of $\approx$1,500 m^2/g.

Mercury porosimetry was used to estimate the macropore volume distribution of the activated carbon. Figure 5.5 shows the cumulative pore volume versus mean pore diameter for F300 activated carbon. Mercury porosimetry measured pore diameters down to 0.008 µm with 0.74 mL/g cumulative volume. The pore volume properties are listed in Table VII.1 (Appendix VII). Also shown in Table VII.1 is the cumulative surface area associated with the internal pore volume. The total surface area estimated from mercury porosimetry (78.6 m^2/g) is substantially less than what was determined by BET nitrogen gas adsorption analysis (570.714 m^2/g). The BET method is more effective for estimating the micropore surface area (where most of the surface area is associated).

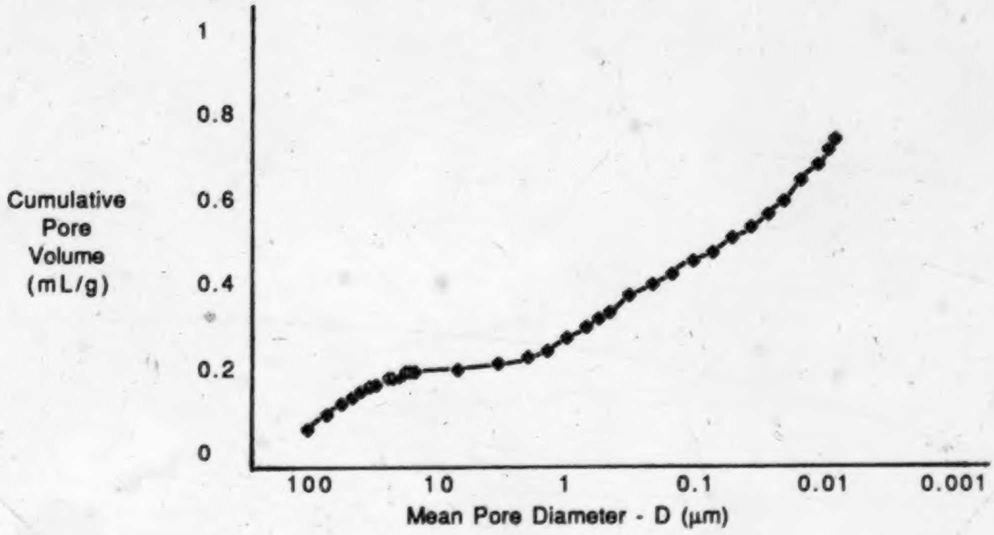


Figure 5.5 Cumulative Pore Volume Versus Mean Pore Diameter for F300 Activated Carbon

Caution must be used in interpreting surface area based on mercury porosimetry measurements. Accurate volume measurements of mercury forced into pores of a material allow the accurate assessment of internal pore volume. Precise pressure measurements required to force the mercury into the pores allow the estimation of the diameter if cylindrical pores are assumed (Faust and Aly, 1983). The total (cumulative) pore surface area can then be estimated by summing the calculated surface areas for all the pores. Error in the surface area will arise when the pores are not cylindrical.

Grunesbeck and Collins (1982) predicted that pore diameters of porous materials should be at least 4 times the diameter of bacteria in order for bacteria to enter. They based their prediction on the study of the entrainment and deposition of bacteria-sized particles in porous media. The average diameters of single-celled bacteria are 0.3-2 µm (Nester et al., 1983). Pores diameters ≥15 µm will be used as the cutoff point below which anaerobic bacteria are not assumed to enter. This pore diameter is larger than the required diameter of averaged-sized bacteria predicted by Grunesbeck and Collins (1982) and Nester et al. (1983) (i.e. 1.2-8 µm). The next lowest mean pore diameter reported during mercury porosimetry measurements was 7 µm (Table VII.1). Although some bacteria may enter a pore with a mean diameter of 7 µm, subsequent growth of biofilm may be limited by the pore size. The choice of the mean pore diameter which bacteria are assumed not to enter (i.e. 7 or 15 μm) is not critical in order to compare the support materials.

The cumulative pore volume of activated carbon associated with mean pore diameter ≥15 μm is 0.192 mL/g (taken as ≥15.13 μm, Table VII.1). This represents the assumed internal volume available for the colonization of bacteria. This also corresponds to an available surface area of 0.0173 m^2/g.

Table VII.1 lists the estimated total surface area down to a mean pore diameter of 0.008 μm as 76.8 m^2/g. The nitrogen gas adsorption (BET) analysis estimated a cumulative surface area of 570.7 m^2/g (Table 5.2) corresponding to a pore diameter of ≈0.001 μm. The internal surface area associated with pore diameters ≥15 μm ($\frac{0.0173}{570.7} \cdot 100\% = 0.003\%$) represents a very small fraction of the total surface area measured by the BET nitrogen gas adsorption method. While this analysis is not exact, it does suggest that very little of the total surface area of activated carbon is available for the colonization of bacteria. Most of the surface area (i.e. >99.99%) of F300 is associated with pore diameters ≤15 μm.

5.1.2 Synthetic Ion Exchange Resins

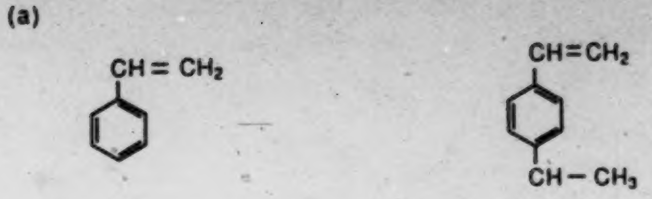
5.1.2.1 Surface Chemistry

Ambergard™ XE-352 and Dowex™ MSC-1 ion exchange resins are comprised of styrene-divinylbenzene (DVB) polymers. The styrene-DVB matrix common to both resins is shown in Figure 5.6. The matrix is a combination of aromatic (benzene) and aliphatic (-CH-CH_2-) structures. This matrix by itself is non-polar and extremely hydrophobic, and would carry a free surface energy that would encourage the adsorption of organic (hydrophobic) compounds to its surface (Kunin, 1980). However, the addition of functional groups with ion exchange sites increases the polarity and greatly reduces the hydrophobicity of the resin. The free surface energy and preference for adsorbed organic compounds are reduced as a result.

The functional group containing the anion exchange site for XE-352 is shown in Figure 5.7a. This functional group is called a "strongly basic" Type I quaternary ammonium group. Quaternary refers to the number of hydrocarbon groups (4) attached to the nitrogen atom. Figure 5.7b shows the "strongly acidic" sulfonic acid functional group containing the cation exchange site for MSC-1.

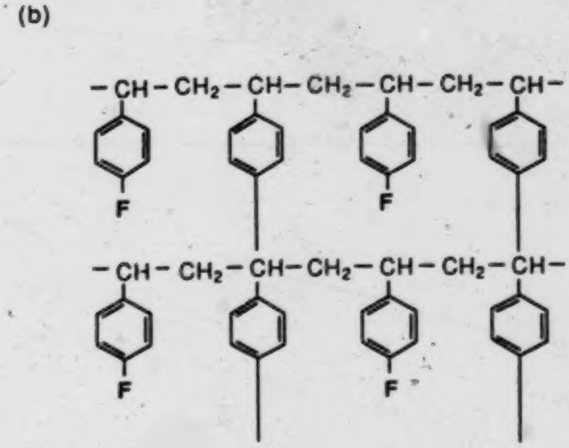
5.1.2.2 **Surface Morphology**

The cumulative pore volume distributions for both ion exchange resins are shown in Figure 5.8. XE-352 has a substantially higher pore volume capacity at pore diameters larger than the assumed cutoff diameter of 15 µm (see section 5.1.1.2). A cumulative volume of 1.220 mL/g was associated with pore diameters ≥15 µm for this resin. The opposite is true of MSC-1. Very little pore volume is present in this resin (0.007 mL/g) at pore diameters ≥15 µm. The pore volume and


(a)
CH= CH2
CH=CH2
CH − CH3

Styrene

Divinyl Benzene (DVB)

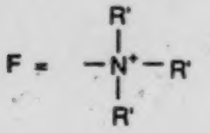

(b)
− CH − CH2 − CH− CH2 − CH− CH2 − CH−
F
F
− CH − CH2 − CH− CH2 − CH − CH2 − CH−
F
F

F = Functional group with ion exchange site.

Cross-Linked Resin Matrix

Figure 5.6 Chemistry of Styrene-DVB Cross-Linked Ion Exchange Resin Matrix (Adapted from Bio-Rad, 1987)

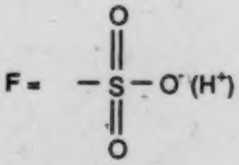
(a) XE-352 (Anion) Exchange Resin



$$F = -\overset{R'}{\underset{R'}{N^+}}-R'$$

R' = Methyl ($-CH_3$) or other hydrocarbon group

(b) MSC-1 (Cation) Exchange Resin



$$F = -\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}-O^-(H^+)$$

Figure 5.7 Chemistry of Functional Groups with Ion Exchange Sites for XE-352 and MSC-1 Synthetic Resins

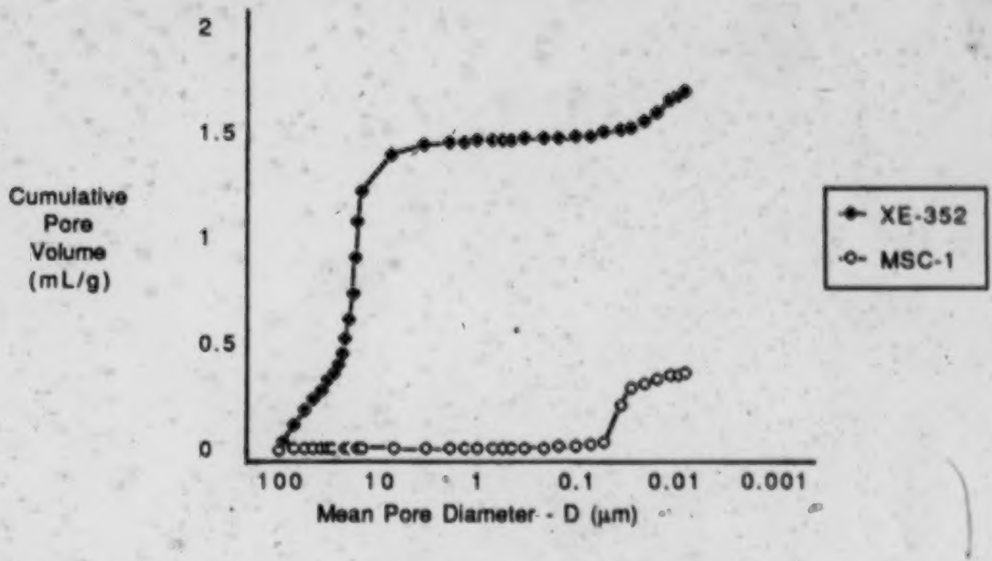


Figure 5.8 Cumulative Pore Volume Versus Mean Pore Diameter Diameter for XE-352 and MSC-1 Synthetic Resins

surface area properties are listed in Table VII.2 (XE-352) and VII.3 (MSC-1) (see Appendix VII).

The surface area associated with pore diameters ≥15 μm is 0.227 m^2/g for XE-352 and 0 m^2/g for MSC-1. The 0 m^2/g surface area of MSC-1 is interpreted as negligible internal surface area. However, the external surface is area is available for bacteria.

5.1.3 Comparison of Support Materials

F300 activated carbon contains phenolic hydroxyl (-OH) groups that comprise the dominant functional species on its surface. Other undefined oxygen groups of less significance are also present. These groups may be associated with oxygen or other impurities listed in Table 5.1. The matrix structure of activated carbon is similar to graphitic carbon (Figure 5.1).

XE-352 resin has a quaternary ammonium ionic group (Figure 5.7a) as the dominant functional species on its surface. MSC-1 has sulfonic acid (Figure 5.7b) as the functional species on its surface. The matrix structure of the synthetic resins is stryene-DVB (Figure 5.6) and differs from activated carbon in that aromaticity is present in the resins.

Pore volume and surface area characteristics of the support materials are summarized in Table 5.3. XE-352 has the largest pore volume of the three materials (6 times the volume of F300 activated carbon and 174 times the volume of MSC-1). Also included in Table 5.3 is the estimated spherical surface area/g for each support material. This parameter was calculated by assuming perfect

Table 5.3 Comparison of Support Materials Based on Pore Volume and Surface Area Characteristics From Mercury Porosimetry

Characteristic	F300 Activated Carbon	XE-352 IX Resin	MSC-1 IX Resin
Available Cumulative Pore Volume (mL/g)[1]	0.192	1.220	0.007
Available Cumulative Surface Area (m^2/g)[1]	0.0173	0.227	0
Total Cumulative Surface Area (m^2/g)[2]	76.8	67.1	57.5
Estimated Spherical Surface Area (m^2/g)[3]	0.0026	0.0085	0.0035
% Area Available For Bacteria[4]	0.022	0.336	0

[1] Associated with mean pore diameters ≥15 µm.

[2] Associated with mean pore diameters ≥0.008 µm.

[3] Assuming spherical particles.

[4] % of estimated total surface area available for colonization of bacteria.

Note: IX = ion exchange.

spheres of an average mean diameter, and using the apparent density as determined by mercury porosimetry. F300 activated carbon had the smallest estimated spherical surface area/g.

An important difference between F300 activated carbon and the ion exchange resins is the adsorptive capacity of activated carbon for organic compounds. Both biodegradable and non-biodegradable organic compounds will have some degree of affinity with the surface of activated carbon. Fluid motion and compound diffusion are two mechanisms that would supply biodegradable organics to the internal pores of all the support materials. Adsorption is an additional mechanism that supplies biodegradable organics to the pores of activated carbon. The adsorption process and related transport of substrate in pores may give activated carbon an advantage for the colonization of bacteria in its pores.

5.2 Characterization of Biomass

Preliminary analyses were performed on samples of anaerobic sludge. The anaerobic sludge contained a mixed population of acclimated phenol-degrading bacteria and was intended as the inoculum for ensuing serum bottle bioassays with the support materials.

The objective of the analysis was to select an acceptable technique for assessing the quantity of anaerobic bacteria. Volatile suspended solids concentration of the anaerobic sludge was used as a benchmark for comparing the reproducibility of each technique.

5.2.1 Organic Nitrogen

Nitrogen is a distinguishing feature of all proteins, accounting for ≈16% of the protein mass (Metcalf and Eddy, 1979). Nester et al. (1983) estimated that elemental nitrogen (components of proteins and nucleic acids) accounts for ≈5.5% of the mass of bacteria. The majority of elemental nitrogen will be in the organic form associated with proteins and nucleic acids. Organic nitrogen analyses of anaerobic sludge were intended to estimate the quantity of anaerobic bacteria.

A triplicate set of nitrogen analysis was performed on the sample of anaerobic sludge. The volatile suspended solids (VSS) concentration of the sludge at the time of analysis was 1,910 mg/L. Table 5.4 shows the results. Ammonia-N concentrations of the sludge were reproducible, however organic-N concentrations were very erratic. The ensuing serum bottle bioassays were designed to be supplemented with 1 mL of anaerobic sludge for a 12.5 mL total liquid volume. The dilution of biomass and associated organic nitrogen, and variable results observed (Table 5.4) would make organic-N a poor measurement of anaerobic biomass.

5.2.2 Carbohydrates

Carbohydrates are an important component in the carbon structure of all organic matter. The carbohydrate content of bacteria can vary from 15 to 35% of the dry cell weight (Gaudy and Gaudy, 1980).

Carbohydrate content in the anaerobic sludge was analyzed several times (Table 5.5). Absorption information at the wavelength

Table 5.4 Ammonia and Organic Nitrogen Concentration of Anaerobic Sludge

	Sample 1	2	3
NH_3-N (mg/L)	41	41	38
Organic-N (mg/L)	160	245	110
VSS (mg/L)[1]	1,910	1,910	1,910
$\frac{\text{Org.-N}}{\text{VSS}}$ Ratio	0.08	0.13	0.06

[1] VSS = Volatile suspended solids.

Table 5.5 Carbohydrate Concentration of Anaerobic Sludge

Trial	Dilution Factor[1]	VSS (µg/mL)[2]	CARB (µg/mL)[3]	$\frac{\text{CARB}}{\text{VSS}}$ Ratio
1	-[4]	1,910	278	0.14
2	-[4]	1,820	262	0.14
3	-[4]	1,790	239	0.13
4	-[4]	3,000	520	0.17
5	-[4]	3,100	604	0.20
6	5	3,310	305	0.09
	10	3,210	300	0.09
	15	3,200	308	0.10
	20	-	324	-

[1] Factor by which anaerobic sludge was diluted with distilled water prior to analysis (dilution of 1 is undiluted).

[2] VSS = Volatile suspended solids.

[3] CARB = Carbohydrates as glucose.

[4] Undiluted.

Note: Results of triplicate analysis performed.
µg/mL = mg/L.

of 485 nm is shown in Appendix VIII for carbohydrates. The results of Table 5.5 indicate the measured carbohydrate content of anaerobic bacteria was not reproducible between different sets of analysis (trials). Carbohydrate/VSS ratios varied from 0.09 (trial #5) to 0.20 (trial #6). The VSS concentration varied from 3,100 to 3,310 mg/L for the same trials.

The experimental technique was consistent and sensitive during an individual trial. The carbohydrate measurements were reproducible during trial #6. The anaerobic sludge was diluted 20-fold (324 µg/mL) and attained similar carbohydrate results to sludge that had only been diluted 5-fold (305 µg/mL).

The use of this measurement technique to assess the biomass associated with support material is questionable. It is well known that biological films on solid supports are not necessarily made up of microorganisms stacked in successive layers. Rather, they consist of cells enmeshed within a matrix of extracellular polymer (Bryers, 1987). The polymer or "glycocalyx" matrix surround the cells and is quite thick (>0.1 µm in most cases) and is composed of polysaccharide or sugar molecules (Costerton, 1984). Carbohydrate analysis of well developed biofilm may over-estimate biomass. This may still occur even if the biomass is physically separated from the solid support prior to analysis because excess portions of the glycocalyx are still associated with detached cells.

5.2.3 **Proteins**

Proteins account for 40-60% of the dry cell mass of bacteria (Gaudy and Gaudy, 1980). A direct protein measurement of anaerobic sludge may represent an estimate of anaerobic biomass. Protein analysis were performed several times on the anaerobic sludge (Table 5.6). Absorption response to the dye-binding compound is provided in Appendix VIII.

Protein analysis were not reproducible between the different sets of analysis performed (Table 5.6). Protein/VSS ratios varied from 0.04 to 0.07. Also protein concentrations were not high (73 µg/mL for trial #2). since the serum bottle bioassays were going to have anaerobic sludge concentations 12.5 times less than what was tested, the resultant protein concentrations may be too low to detect biomass.

5.2.4 **Discussion of Characterization Methods**

Organic nitrogen, carbohydrate, and protein analyses were not adequate for the measurement of biomass quantities in the ensuing serum bottle bioassays because of the low initial biomass concentration and low liquid volumes planned.

An alternate procedure was used by measuring the phenol removal rate as a measure of phenol-degrading activity of biomass in the serum bottles. This approach did not allow the direct assessment of the quantity of biomass associated with the different support materials, however, it did allow comparison of biomass activity associated with the materials.

Radiolabelled cell adsorption studies and scanning electron microscopy (SEM) observations were done to provide additional information on biomass associated with the support materials. The cell adsorption studies provided information on initial adsorption

P

Table 5.6 Protein Concentration of Anaerobic Sludge

Trial	VSS (µg/mL)[1]	Protein (µg/mL)[2]	$\frac{\text{Protein}}{\text{VSS}}$ Ratio
1	1,910	100	0.05
2	1,820	73	0.04
3	1,790	85	0.05
4	3,000	200	0.07

[1] VSS = Volatile suspended solids.

[2] Protein as bovine plasma gamma gobulin (BPGG).

Note: Results of triplicate analysis performed.
µg/mL = mg/L.

and attachment of bacteria to the material surface. SEM was used to observe biomass growth on the surface.

5.3 Characteristics of Phenol Adsorption on F300 Activated Carbon

The phenol adsorption characteristics of F300 activated carbon were investigated. Short term (2 h) kinetic data and longer term (5 day) equilibrium information was obtained in order to assess the interaction of physical/chemical adsorption with anaerobic biodegradation of phenol.

5.3.1 Kinetics of Phenol Adsorption

The kinetics of phenol adsorption on F300 activated carbon was observed in a batch (serum bottle) reactor under anaerobic conditions.

5.3.1.1 Procedures

The kinetic test was performed in a 58 mL volume serum bottle. The bottle was prepared according to the procedure described in section 3.1.1 with one exception. After flushing the bottle and prior to the addition of the liquid contents, 75 mg of F300 activated carbon (420-850 µm) was weighed out and added to the bottle. The bottle received 11 mL of a growth medium mixture and 1 mL synthetic growth medium (Table 3.2).

The growth medium mixture contained 70% synthetic growth medium (Table 3.2) and 30% digestor supernatant. The digestor supernatant was obtained from an anaerobic digestor of a local municipal wastewater treatment plant. The supernatant was allowed to settle in sealed plastic containers for 24 h at 4°C. The upper portion of the supernatant was transferred anaerobically to 158 mL serum bottles (100 mL per serum bottle). After sealing, the bottles were autoclaved for 20 min at 121°C. The supernatant (70%) and synthetic medium (30%) were anaerobically added to flushed 119 mL serum bottles and sealed. This growth medium mixture was stored at 4°C until used.

The 58 mL serum bottle containing 75 mg activated carbon, 11 mL growth medium mixture, and 1 mL synthetic medium was sealed and incubated anaerobically for 24 h (at 37°C) on a shaker table (175 RPM). The bottle was given 0.5 mL of a reduced phenol stock solution (13,100 mg/L) and phenol analyses were immediately performed (section 3.2.6). Aqueous phenol concentations were analyzed over a period of 2 hours. The bottle was continuously shaken (175 RPM) at 37°C during this time.

5.3.1.2 **Results and Discussion**

The adsorption rate of phenol on activated carbon is shown in Figure 5.9. The initial phenol concentration in the serum bottle was 525 mg/L. Rapid uptake of phenol occurred over the first 33 min, aqueous phenol concentrations decreased from 525 to less than 73 mg/L during this time. Phenols levels were reduced from 73 to 37 mg/L over the next 87 min.

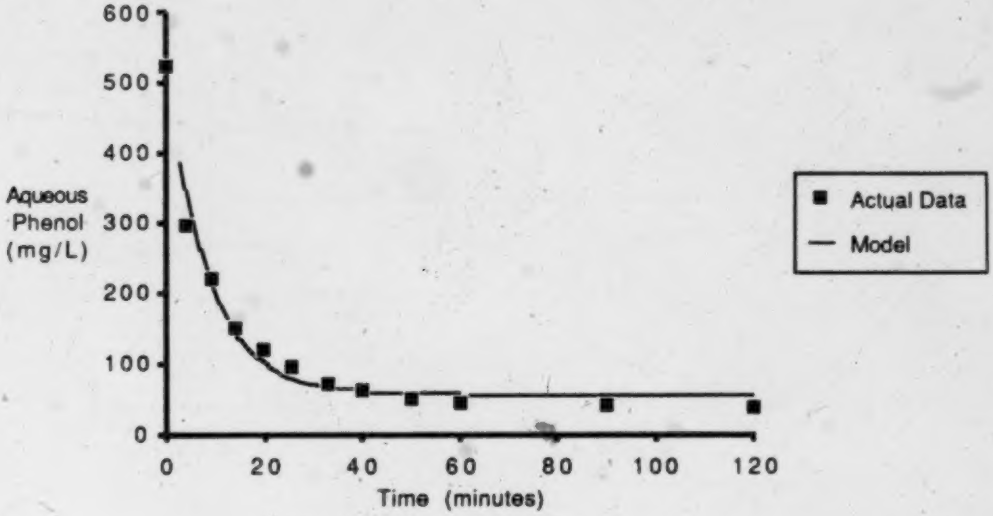


Figure 5.9 Phenol Adsorption Rate on F300 Activated Carbon Prior to the Addition of Bacteria

The phenol adsorption rate was assumed to be 1st order with respect to time. The 1st order rate equation describing phenol adsorption to activated carbon in a batch reactor can take on the form:

$$V\left[\frac{dC_{(t)}}{dt}\right] = -kW[C_{(t)} - C^{*}], \text{ where:} \qquad \text{(Eqn. 5-2)}$$

V = liquid volume of reactor (0.0125 L),

W = mass of activated carbon (0.075 g),

k = apparent 2 h reaction rate constant $(\frac{L}{g \cdot min})$,

C^{*} = 2 h quasi-equilibrium phenol concentration (mg/L),

$C_{(t)}$ = phenol concentration at time t = t, and

t = time (min).

Integrating equation (5-2) between t=0 and t=t and solving for $C_{(t)}$ gives:

$$C_{(t)} = [C_{(o)} + C^{*}]e^{-kWt/V} + C^{*}, \qquad \text{(Eqn. 5-3)}$$

where $C_{(o)}$ = phenol concentration at time t=0 (525 mg/L). Equation (5-3) allows the prediction of the phenol adsorption rate when V or W is changed. A non-linear least squares method was used to fit the rate data to the model (equation 5-3) yielding:

$$C_{(t)} = 470e^{-t/8.224} + 55. \qquad \text{(Eqn. 5-4)}$$

Therefore:

$$k = \frac{0.0125\ \text{L}}{8.224\ \text{min}(0.075\ \text{g})} = 0.0203 \frac{\text{L}}{\text{g}\cdot\text{min}}, \qquad \text{(Eqn. 5-5)}$$

(95% confidence interval: $0.0166 \leq k \leq 0.0239$), and

$C^{*} = 55$ (mg/L),

(95% confidence interval: $37 \leq C^{*} \leq 73$).

C^{*} can be described further to allow examination of the phenol adsorption capacity of activated carbon. Let q represent the mass loading of phenol adsorbed to activated carbon:

$$q = \frac{\text{mass adsorbed phenol}}{\text{mass activated carbon}}, \text{ therefore:} \qquad \text{(Eqn. 5-6)}$$

q^{*} = quasi-equilibrium mass loading based on a 2 h (120 min) contact time.

A mass balance of phenol before and 2 h after adsorption is:

$$VC_{(o)} = VC^{*} + Wq^{*}. \qquad \text{(Eqn. 5-7)}$$

Solving equation (5-7) for C^{*} gives:

$$C^{*} = C_{(o)} - \frac{Wq^{*}}{V}. \qquad \text{(Eqn. 5-8)}$$

Substituting equation (5-8) into equation (5-3) and rearranging:

$$C_{(t)} = [2C_{(o)} - \frac{Wq^{*}}{V}]e^{-kWt/V} + [C_{(o)} - \frac{Wq^{*}}{V}]. \qquad \text{(Eqn. 5-9)}$$

The mathematical statement in equation (5-9) expresses the the phenol adsorption behavior in terms of the rate constant k and quasi-equilibrium constant q^{*} and allows prediction of the adsorption behavior when V or W is changed.

Equation (5-8) can be rearranged and solved for q^{*}:

$$q^* = [C_{(o)} - C^*] \frac{V}{W}. \qquad \text{(Eqn. 5-10)}$$

Substituting $C_{(o)}$ (525 mg/L), C^* (55 mg/L), W, and V into equation (5-10) gives:

$$q^* = [525 - 55] \frac{mg}{L} * \frac{0.0125\ L}{0.075\ g} = 78.3 \frac{mg}{g}, \qquad \text{(Eqn. 5-11)}$$

(95% confidence interval: $75.3 \le q^* \le 81.3$).

The apparent rate constant k (equation 5-5) and quasi-equilibrium constant q^* (equation 5-11) represent the expected behavior of phenol adsorption to virgin activated carbon in the absence of anaerobic bacteria. This information will eventually be compared to phenol adsorption in the presence of anaerobic bacteria (attached to the surface of activated carbon) after the activated carbon has been repeatedly loaded with phenol (section 5.4.1.2).

5.3.2 Phenol Adsorption Equilibria

The adsorption equilibria of F300 activated carbon was investigated by measuring the phenol uptake capacity for different activated carbon concentrations. The equilibrium data was analyzed and interpreted according to the Freundlich isotherm equation.

5.3.2.1 Procedures

The isotherm experiment was performed in 58 mL volume serum bottles. The bottles were flushed according to the procedure described in section 3.1.1. F300 activated carbon doses of 0, 9.6, 24.9, 34.6, 50.0, 75.3, and 80.0 mg were added to separate bottles. Growth medium mixture (11 mL - see section 5.3.1.1), and synthetic medium (1 mL - see Table 3.2) were anaerobically added to each bottle. After sealing, the bottles were allowed to equilibrate at 37°C on a shaker table (175 RPM) for 48 h.

Each bottle was given 0.5 mL of a reduced phenol stock solution (12,930 mg/L) and set back on the shaker table. Aqueous phenol concentations were analyzed after 5 days.

5.3.2.2 **Results and Discussion**

The adsorption isotherm for phenol is shown in Figure 5.10. The Freundlich isotherm describing the adsorption equilibria takes on the general form:

$$q = kc_e^{1/n}, \qquad \text{Eqn. (5-12)}$$

where:

$q =$ mg adsorbed phenol/g activated carbon,
$k =$ adsorption coefficient (L/g),
$c_e =$ equilibrium phenol concentration (mg/L), and
$n =$ equation constant.

The parameter k is a measure of the phenol loading capacity on activated carbon. Higher k values indicate a higher loading of adsorbed phenol on activated carbon. A higher $\frac{1}{n}$ value indicates greater sensitivity in phenol loadings.

The fitted Freundlich isotherm equation for phenol adsorption to F300 activated carbon is shown in Table 5.7.

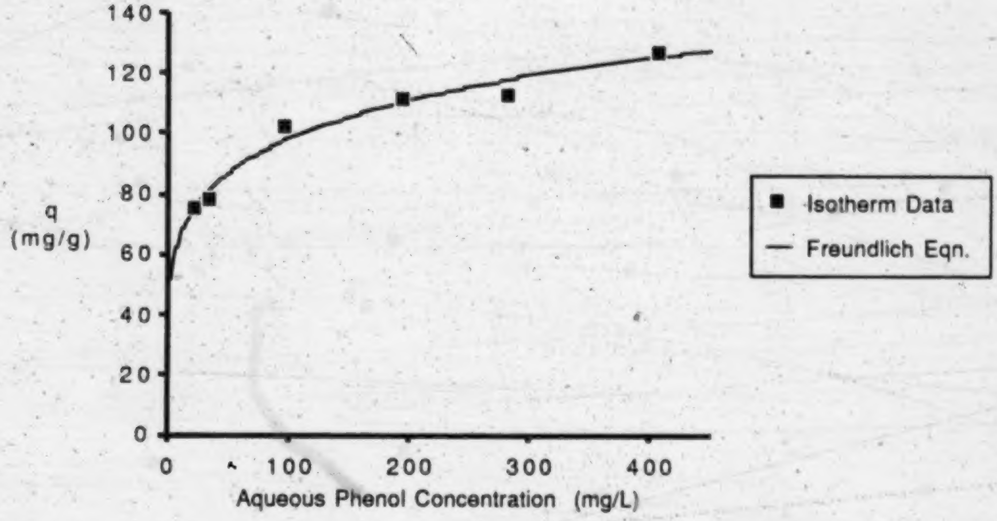


Figure 5.10 Adsorption Isotherm of Phenol Fitted to the Freundlich Equation

Table 5.7 Published Data for Phenol Adsorption Isotherms

Activated Carbon Type	Particle Size (µm)	Time Period (days)	Test Conditions	Freundlich Equation	Reference
F300	420-850	5	37°C, pH 7 growth medium	$q = 44.4c_e^{0.17}$ (*)	present study
F300	<75 µm	5	20°C	$q = 21c_e^{0.54}$	Dobbs and Cohen (1980)
F400	600-1,190	23-40	20°C, pH 7	$q = 78.1c_e^{0.212}$	Peel and Benedek (1980)
F400	500-600	6-42	20°C,	$q = 56c_e^{0.288}$	"
F400	420-1,190	2-8	25°C	$q = 68c_e^{0.19}$	"

(*) 95% confidence interval for $0 \le c_e \le 400$ mg/L:

$37.3 \le k \le 51.5$, $0.15 \le \frac{1}{n} \le 0.20$.

Also listed in Table 5.7 are experimentally fitted Freundlich equations for phenol adsorption to F300 and F400 activated carbon (summarized from Dobbs and Cohen, 1980; and Peel and Benedek, 1980). It is not appropriate to compare k (or $\frac{1}{n}$) individually with other reported values because k and $\frac{1}{n}$ are correlated. Table 5.7 provides a comparison of the Freundlich equations which include both k and $\frac{1}{n}$.

5.4 Phenol Fermentation Studies with Biological Supports

Semicontinuous biological assays were designed to observe anaerobic degradation of phenol in the presence of solid support materials (activated carbon and synthetic ion exchange resins). The objective of this study was to try identify features of the microbe-biological support system that provide enhanced removal and eventual conversion of phenol to methane.

Serum bottles containing the different support materials in a suitable anaerobic growth medium were supplemented with phenol and a suspension of acclimated phenol-degrading anaerobic bacteria. After allowing time for biological attachment to and growth on the solid supports, the cultures had most of their supernatant removed and replaced with a suitable growth medium free of bacteria. This was performed to remove suspended bacteria from the cultures. The remaining attached organisms were then monitored for their ability to remove phenol. The activity of the cultures was compared by determining the rate of phenol removal. Direct measurements of biomass organic nitrogen, carbohydrate, and protein were not adequate for comparing the biomass quantity associated with low concentrations of anaerobic bacteria (section 5.2).

5.4.1 Activated Carbon

5.4.1.1 Procedures

a) The bioassay was performed in 58 mL volume serum bottles. Three bottles (designated A, B, and C) were prepared according to the procedure described in section 3.1.1 with one exception. After flushing the bottles and prior to the addition of the liquid contents, 75 mg of F300 activated carbon (420-850 µm) was weighed out and added to each bottle. This corresponded to an activated carbon concentration of 6,000 mg/L. The bottles received 10.4 mL of the growth medium mixture (see section 5.3.1.1). The bottles were capped, sealed, and given 0.1 mL of a sulfide solution (see Table 3.1) and 12.2 mg phenol (i.e. 1 mL of a phenol stock solution 12,200 mg/L). The bottles were agitated on a shaker table (175 RPM) in an incubator (37°C) for 5 days to allow phenol to equilibrate between the solution and activated carbon phase. 1 mL of a suspension of acclimated phenol-degrading consortium was added to each bottle (12.5 total liquid volume). The removal of phenol and biological methane production were monitored.

b) The cultures were allowed to remove phenol to concentrations less than 50 mg/L. At this point the cultures were either supplemented with phenol from a reduced stock solution, or, they had 88% of the liquid contents removed and replaced with a bacteria-free medium prior to phenol supplementation.

The purpose of removing 88% of the supernatant was to minimize or remove suspended bacteria from the serum bottles. This corresponded to removing 11 mL of the total 12.5 mL liquid volume, and replacing it with 10 (or 10.5) mL of the growth medium mixture (see section 5.3.1.1) and 1 (or 0.5) mL of reduced phenol stock solution (12,200 mg/L). The 1 (or 0.5) mL addition of stock solution resulted in the theoretical addition of 12.2 (or 6.1) mg of phenol to the serum bottles.

c) The supernatant exchange procedure was accomplished in the following manner. The biological gas was allowed to accumulate in the bottle headspace in excess of the exchange volume (11 mL). This ensured a positive pressure in the bottles during the exchange process and minimized oxygen contamination. The bottles were taken from the shaker table, inverted, and shaken by hand to completely mix the contents. As soon as the carbon particles began settling (≈15 s), a flushed needle and syringe was inserted through the rubber stopper above the settling carbon particles. 11 mL of the medium suspension was rapidly removed. The bottles were positioned upright and another flushed needle and syringe was used to inject the growth medium mixture into the bottles. The bottles were supplemented with the reduced phenol stock solution and placed on the shaker table.

After 60 min, the bottles were analyzed for aqueous phenol. The 60 min interval represented the period of time in which primarily physical/chemical adsorption of phenol was assumed to occur. This time period was estimated from the kinetic phenol adsorption characteristics in Figure 5.9. Phenol concentrations in Figure 5.9 had levelled out after 60 min, with only minor reductions over the next 60 min.

d) The phenol-degrading activity of the suspended bacteria removed from the bottles containing actived carbon was also of interest. The 11 mL supernatant removed from each bottle was used to start two new cultures. One culture received 1.5 mL of the 11 mL as its inoculum, and the other culture received the remaining 9.5 mL as its inoculum. These cultures were initially prepared similar to the activated carbon cultures and had the following components:

i) 1.5 mL inoculated culture:
  - 1.5 mL of suspension withdrawn from activated carbon cultures,
  - 10.5 mL growth medium mixture, and
  - 0.5 mL reduced phenol stock solution (12,200 mg/L).

ii) 9.5 mL inoculated culture:
  - 9.5 mL of suspension withdrawn from activated carbon cultures,
  - 2.5 mL growth medium mixture, and
  - 0.5 mL reduced phenol stock solution (12,200 mg/L).

The cultures were designated 12% (for the 1.5 mL inoculum) and 76% (for the 9.5 mL inoculum) because they represented the percent liquid volume removed from the original 12.5 mL activated carbon culture. The 12% cultures provided a direct comparison of suspended bacteria to the activated carbon culture (which had 1.5 mL, or 12%, of the original liquid volume remaining).

e) The schedule of operations performed on the triplicate cultures (A, B, and C) containing activated carbon is summarized in Table 5.8 The cultures were maintained for approximately 100 days.

f) The phenol adsorption kinetics were measured in culture B on day 91. The bottle was given 0.5 mL of a reduced phenol stock solution (13,100 mg/L) and aqueous phenol concentations were analyzed over a period of 2 h. The bottle was continuously shaken (175 RPM) at 37°C during this time.

5.4.1.2 **Results and Discussion**

The behavior of cultures A, B, and C with respect to phenol removal is displayed in Figure 5.11. All three cultures exhibited similar behavior during the first 55 days incubation, therefore the average aqueous phenol concentrations of the three cultures is shown in Figure 5.11. The bottles (minus bacteria) were initially given 12.2 mg phenol on day -5 and allowed to equilibrate. The cultures were inoculated with

Table 5.8 Operation Schedule for Activated Carbon Bioassay

Incubation Day	Operation Performed
-5	Serum bottle setup
0	Bottles inoculated with bacteria
7	Fed 1 mL phenol
20	S.E., fed 1 mL phenol
41	Fed 0.5 mL phenol
51	S.E., fed 0.5 mL phenol
60	Fed 0.5 mL phenol
66	S.E., fed 0.5 mL phenol
72	Fed 0.5 mL phenol

Cultures operated individually after day 75:

A	B	C	
79	75	76	S.E., fed 0.5 mL phenol, 1st S.N.C.
84	78	83	S.E., fed 0.5 mL phenol, 2nd S.N.C.
90	82	88	S.E., fed 0.5 mL phenol, 3rd S.N.C.
98	86	92	S.E., fed 0.5 mL phenol, 4th S.N.C.

phenol - Reduced phenol stock solution (12,200 mg/L).

S.E. - Supernatant exchange procedure whereby 88% (11mL) of the activated carbon culture supernatant was removed and replaced with reduced bacteria-free growth medium.

S.N.C. - Setup of new cultures using supernatant (removed from activated carbon cultures) as inoculum.

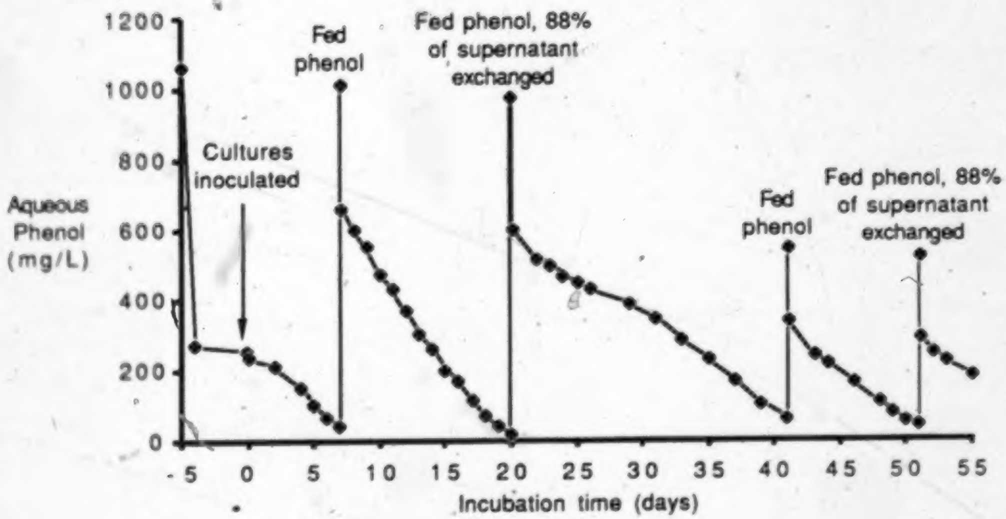


Figure 5.11 Aqueous Phenol Levels in Anaerobic Cultures Containing 6,000 mg/L F300 Activated Carbon

bacteria on day 0 and supplemented with 12.2 mg phenol on day 7 and day 20. Just prior to phenol supplementation on day 20, the cultures had 88% of the supernatant removed and replaced with biomass-free liquid. The cultures were fed 6.1 mg phenol on demand on day 41 and 51. All subsequent demand feedings after day 20 were 6.1 mg phenol. The cultures were analyzed for phenol 60 min after supplementation, and daily thereafter.

Figure 5.11 clearly demonstrates the reduction in adsorption capacity of activated carbon due to the presence of bacteria and previous phenol loading. The addition of 12.2 mg phenol on day -5 resulted in 2.9 mg remaining in solution (9.3 mg adsorbed) before the cultures were inoculated. The next addition of 12.2 mg phenol on day 7 saw 8.3 mg phenol in the effluent after 60 min (only 3.9 mg adsorbed).

The effect of removing and replacing 88% of the supernatant on day 20 is evident. From day 8 to day 19 the aqueous phenol removal rate averaged 51 mg/L·d. After exchanging the supernatant on day 20, the removal rate was 24 mg/L·d (averaged from day 22 to 39). Removing 88% of the liquid suspension only reduced the biomass activity by 53%. The remaining biomass activity would be primarily associated with surface-attached bacteria, and with suspended bacteria in the remaining 12% liquid suspension.

The expected and observed methane production for the cultures are shown in Figure 5.12. The expected methane volume was determined by calculating the methane equivalent of phenol added just prior to the next phenol supplementation

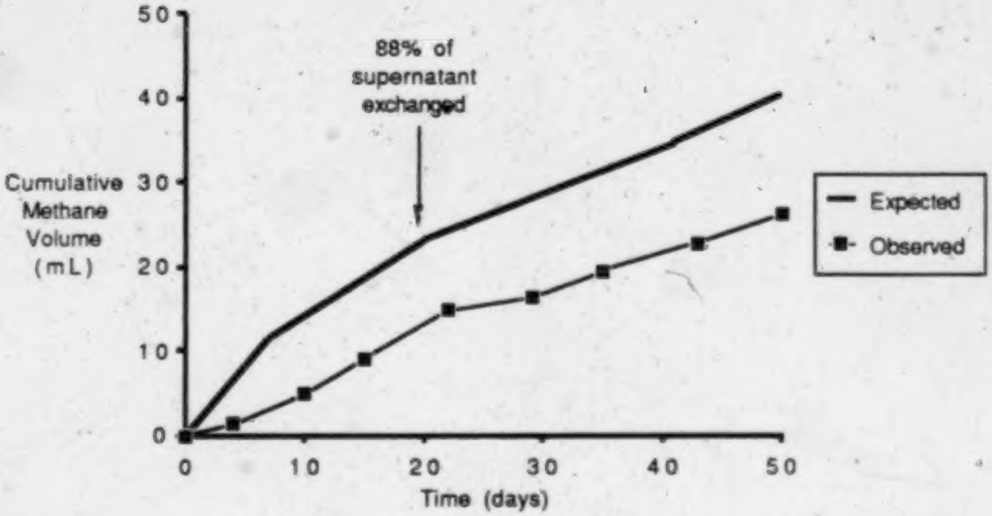


Figure 5.12 Expected and Observed Methane Production of Anaerobic Cultures Containing 6,000 mg/L F300 Activated Carbon

episode. For example, the expected methane volume up to day 7 (just prior to the addition of 12.2 mg phenol) would be calculated from the 12.2 mg phenol added on day -5. The expected methane volume on day 40 (just prior to the addition of 6.1 mg phenol) would be the methane equivalent of 12.2 mg of phenol added on day -5, 7, and 20 (i.e. 12.2+12.2+12.2 = 36.6 mg). The methane equivalent of phenol, assuming complete conversion at 37°C and 1 atm, is:

$$1 \text{ mg phenol} = 0.95 \text{ mL } CH_4.$$

The methane equivalent of phenol is calculated from the COD equivalent of phenol (Appendix II):

$$1 \text{ mg phenol} = 2.38 \text{ mg COD, and}$$

the methane equivalent of COD (Equation VI-5, Appendix VI):

$$1 \text{ mL } CH_4 = 2.5 \text{ mg COD.}$$

The observed methane production was less then the expected production. The difference in expected and observed methane was assumed to be associated with phenol that was adsorbed to activated carbon and presumed to be unavailable to bacteria during the period measurements were made, and to phenol that was biodegraded and incorporated as cell biomass. The adsorbed phenol would eventually be available for biological conversion upon desorption from the activated carbon surface.

The percentage of expected methane observed is compared to the percentage of added phenol that remains in solution on day 7, 20, and 42 in Table 5.9. A direct comparison is possible because the expected methane production is based on the

Table 5.9 Methane Production in Cultures Containing F300 Activated Carbon

Time[1]	% $\frac{CH_4 \text{ Observed}}{CH_4 \text{ Expected}}$	% $\frac{\text{Phenol in Solution}}{\text{Phenol Added}}$
7	25	24
20	56	46
42	63	51

---

[1] Incubation time (days) prior to next phenol supplementation episode.

amount of phenol added to the cultures. The phenol remaining in solution represents the amount not adsorbed to the activated carbon, and therefore available for conversion to methane (i.e. the fraction of phenol that was not adsorbed during the first 60 min). This amount was measured on day 0 (before inoculation), and 60 min after each phenol supplementation episode thereafter. For example, up to day seven 12.2 mg of phenol was added (on day -5) and after equilibration with the activated carbon had 232 mg/L in solution. This represents 2.9 mg phenol in solution (per 12.5 mL liquid volume) and 9.3 mg adsorbed to the carbon. The percentage of added phenol in solution was $\frac{2.9}{12.2} * 100\% = 24\%$. The percentage of expected methane observed was 25% for this same period. This demonstrates that all of the phenol remaining in solution was converted to methane up to day 7 and little or no adsorbed phenol was converted to methane during this time. However, the data reported up to day 20 and 42 (Table 5.9) clearly indicate that phenol, in excess of the amount measured in solution after 60 min, was being converted to methane. Up to day 20, 56% of the expected methane was observed, while only 46% of the added phenol was available in solution to bacteria after 60 min. The excess methane production up to day 20 may have been from phenol that was initially adsorbed to the activated carbon and later desorbed and converted to methane.

From day 55 to day 75 the cultures were fed phenol according to the schedule in Table 5.8, however the aqueous phenol concentration and methane produced was not monitored. During this time 88% of the supernatant was exchanged twice (on day 51 and 66). After day 75 the cultures were monitored individually and were each subjected to four consecutive supernatant exchanges (Table 5.8). The supernatant removed from the cultures was used to start new cultures to measure the supernatant activity. The behavior of aqueous phenol in culture B and in the cultures containing 12 and 76% of the supernatant removed from culture B during the four consecutive supernatant exchanges is shown in Figures 5.13a to 5.13d. The behavior of aqueous phenol in cultures A and C during this phase is shown in Figures IX.1 and IX.2 (Appendix IX).

All cultures were theoretically given 6.1 mg phenol at each feed episode. This mass of phenol is equivalent to an aqueous concentration of 490 mg/L. The activated carbon cultures exhibited the most rapid decrease in aqueous phenol concentration in all instances. These cultures were able to completely remove phenol from solution in 3 to 5 days due to rapid initial adsorption and biodegradation. Measurements of methane in all activated carbon cultures were stopped by this time because the supernatant exchange procedures resulted in the accumulated loss of biological gas from the serum bottles. However, the behavior of aqueous phenol concentrations in cultures A, B, and C during all consecutive supernatant

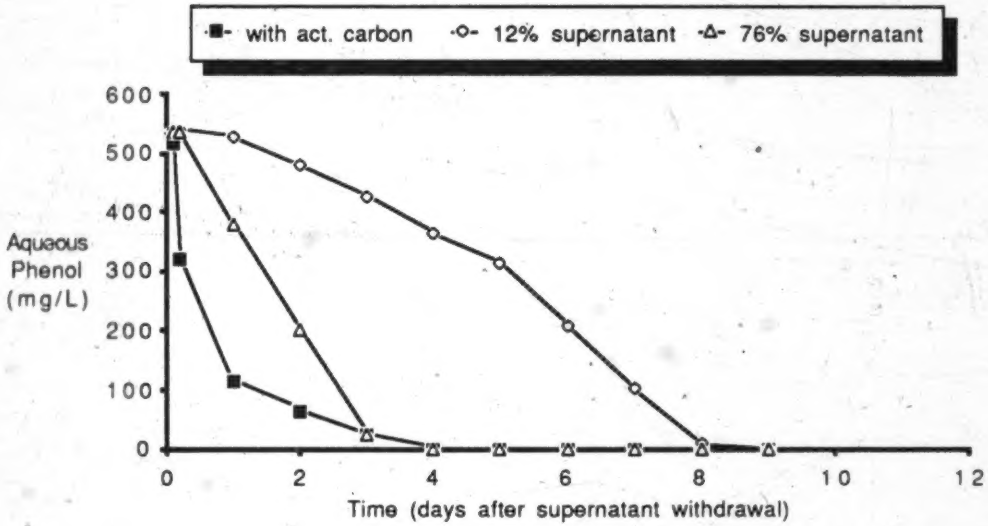


Figure 5.13a Phenol Biodegradation in Culture B with F300 Activated Carbon and in Supernatant Removed From Culture B (1st consecutive supernatant withdrawal)

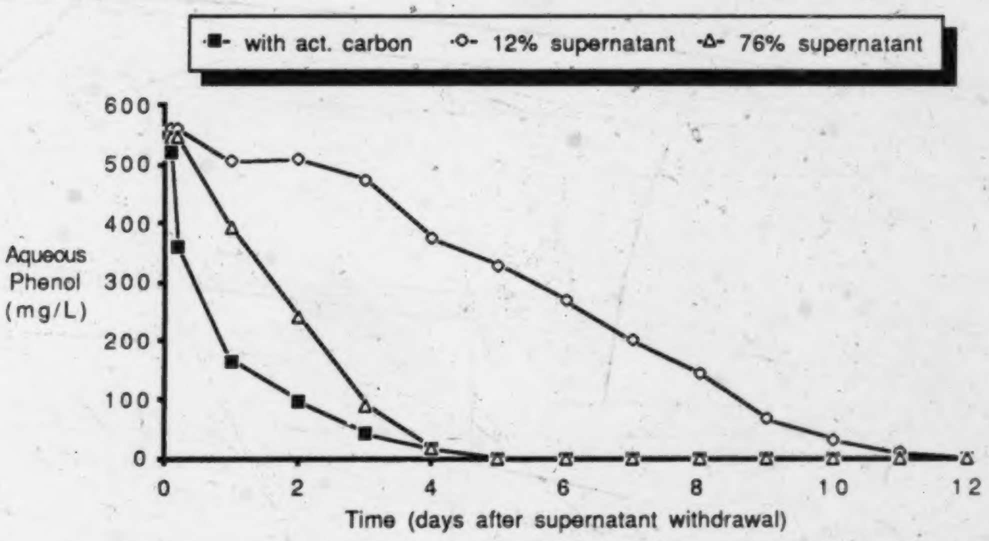


Figure 5.13b Phenol Biodegradation in Culture B with F300 Activated Carbon and in Supernatant Removed From Culture B (2nd consecutive supernatant withdrawal)

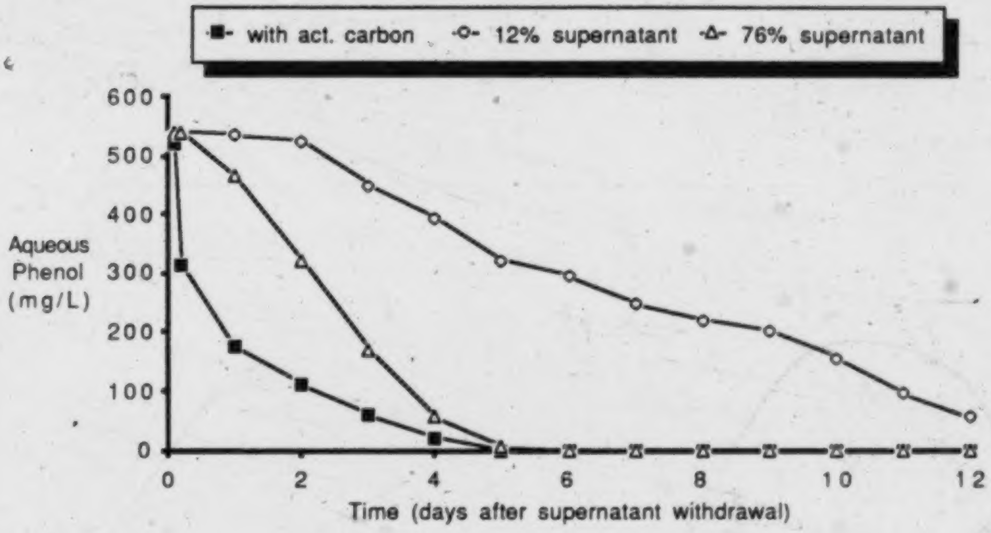


Figure 5.13c Phenol Biodegradation in Culture B with F300 Activated Carbon and in Supernatant Removed From Culture B (3rd consecutive supernatant withdrawal)

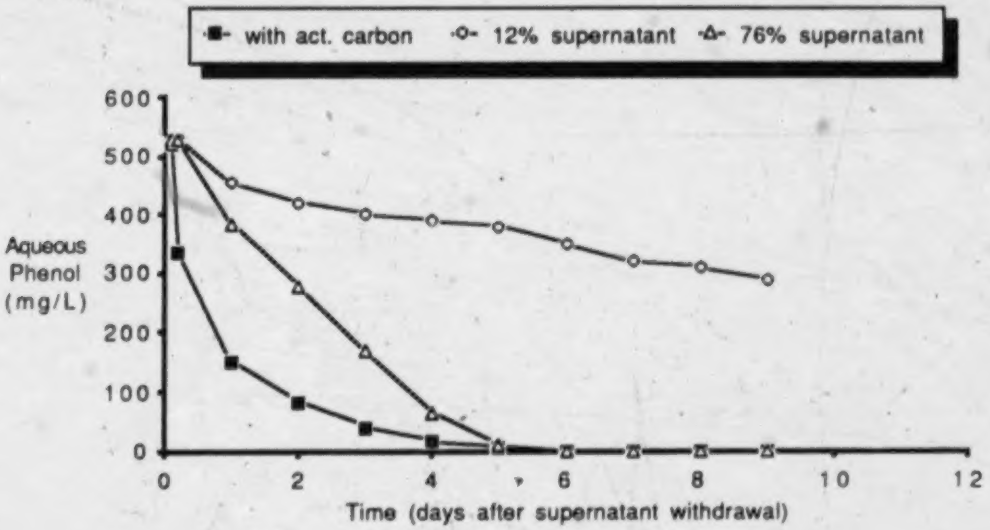


Figure 5.13d Phenol Biodegradation in Culture B with F300 Activated Carbon and in Supernatant Removed From Culture B (4th consecutive supernatant withdrawal)

exchanges strongly suggest that bioregeneration was occurring throughout.

The aqueous phenol concentration in cultures A, B, and C was measured 60 min after the addition of 6.1 mg phenol, following each supernatant exchange. The 60 min aqueous phenol concentration for each culture is shown in Table 5.10. An interesting feature of Table 5.10 is that in each situation a similar amount of phenol remained in solution (336 ± 22 mg/L) after 60 min. This mean concentration is equivalent to 4.2 mg or 69% of the original 6.1 mg phenol added. More importantly, a similar amount of phenol (1.9 mg) was adsorbed to the carbon after 60 min each time 6.1 mg phenol was added. This seems to suggest that the same phenol adsorption capacity was available each time the cultures were fed phenol. In essence, the same amount of bioregeneration was occurring each time. The operating conditions appear to be well suited for bioregeneration. That is to say, cycles of initial phenol adsorption followed by desorption under conditions of reduced aqueous phenol concentrations were occurring (Kim et al., 1986).

The actual phenol biodegradation rate occurring in the carbon cultures is a combination of the rate of phenol removal from aqueous solution, and, phenol removal associated with activated carbon desorption. Because a similar amount of phenol was adsorbed to the carbon each time, it is reasonable to assume a similar amount of phenol was desorbed and biodegraded each time. The average phenol biodegradation rate

Table 5.10 Aqueous Phenol Concentration in Cultures Containing F300 Activated Carbon After 60 Minutes Equilibration (mg/L)

Consecutive Supernatant Withdrawal	Culture		
	A	B	C
1st	315	322	328
2nd	335	360	375
3rd	320	315	312
4th	350	335	370

Mean = 336 mg/L (or 4.2 mg per 12.5 mL liquid volume).
S.D. = 22 mg/L.

Note: The mean aqueous phenol concentration is equivalent to 4.2 mg (or 69%) of the original 6.1 mg added phenol.
S.D. = standard deviation.

can be approximated from: the phenol in solution at time 0 (based on addition of 6.1 mg), and the aqueous concentration just prior to re-feeding (taken at 4 days after supernatant withdrawal in most instances). The estimated phenol biodegradation rate during the first 4 days after each supernatant withdrawal in cultures A, B, and C is shown in Table 5.11.

It is interesting to note that the estimated phenol biodegradation rate did not decrease with subsequent supernatant withdrawals. This can be compared to the first supernatant withdrawal in cultures A, B, and C when the observed phenol biodegradation rate decreased from 51 to 24 mg/L·d (Figure 5.11). The attached-growth bacteria had developed to a greater degree by the time consecutive supernatant withdrawals were performed (i.e. after 75 days incubation).

A phenomenon that was repeatedly observed in the cultures containing 76% supernatant was their high capacity for phenol removal. These cultures which were free of activated carbon were able to completely remove phenol from solution via biodegradation in 4 to 5 days. This was observed for each of the consecutive supernatant exchanges (Figures 5.13a to d, IX.1a to d, and IX.2a to d). Table 5.11 shows the phenol biodegradation rate for these cultures (calculated between the first and third day after supernatant withdrawal).

Table 5.11 Biological Phenol Removal Rate of Cultures During Activated Carbon Assay

Consecutive Supernatant Withdrawal	Phenol Removal Rate (mg/L·d)	
	With Act. Carbon	76% Supernatant
Culture A:		
1st	122	183
2nd	121	185
3rd	97	119
4th	113	111
Culture B:		
1st	130	189
2nd	126	152
3rd	125	148
4th	126	109
Culture C:		
1st	114	143
2nd	122	145
3rd	119	139
4th	110	179
Mean =	119	150
S.D. =	9	39

Note: S.D. = standard deviation.
Removal rate for activated carbon cultures calculated from day 0 (i.e. after addition of 6.1 mg phenol) to 3 days after supernatant withdrawal.
Removal rate for 76% supernatant cultures calculated between 1 and 3 days after supernatant withdrawal.

Activated carbon appeared to rapidly transfer the phenol degradation capability (i.e. phenol-degrading bacteria) into the supernatant. The average phenol removal rate in cultures containing 76% supernatant (150 ± 39 mg/L·d) was more rapid than the activated carbon cultures containing attached-growth organisms (119 ± 9 mg/L·d). If activated carbon did not transfer any phenol-degrading bacteria into the supernatant, the four consecutive supernatant exchange procedures would eventually wash out 99.98% of the phenol-degrading bacteria originally in the 12.5 mL supernatant of the cultures containing activated carbon (prior to the first consecutive supernatant exchange). However, Table 5.11 demonstrates that the phenol removal rates in the 76% supernatant cultures (4th supernatant exchange) were as or more efficient than the cultures containing activated carbon. The 76% supernatant cultures from A and B did show a decreasing trend in their rate of phenol removal with subsequent supernatant exchanges. This trend was not observed with the C culture.

The repetitive observation of high bioactivity in the 76% supernatant cultures agrees well with the work of Chang and Rittmann (1988). The authors studied the formation of aerobic biomass on activated carbon surfaces. A continuous flow reactor was fed phenol and observed over a 110 h period. They observed negligible biomass loss from the activated carbon surface in the first 40 h. However, the biomass loss increased suddenly over the next 10 h and was constant for the next 60 h. The bioactivity in the 76% supernatant cultures would be primarily from biomass transferred from activated carbon-attached biomass (in the activated carbon cultures).

The loss of biomass from the activated carbon surface may have been related to:

i) hydraulic shear,
ii) abrasion of the support material, or
iii) sloughing of attached-biomass from the support surface.

Hydraulic shear is responsible for the continuous, fluid-dynamic dependent removal of small areas of the biomass from the support surface (Stronach et al., 1986). Mixing and agitation of the reactor contents causes the support materials to collide and the resulting abrasion will continuously remove excess biomass (Bryers, 1987). Sloughing refers to the random, bulk film detachment of biomass, and is usually considered to be a result of depletion of nutrients deep within the biomass film or some significant environmental changes (Stronach et al., 1986). Bryers and Characklis (1982) found that biomass loss from solid supports occurred continuously throughout biological film formation and increased with increasing Reynolds number.

Speitel et al. (1987) modelled the shearing of biomass in packed-bed GAC columns operated under dynamic substrate loading conditions. Their modelling results indicated that biomass shearing was related to the microbial growth rate. The substrate feeding episodes and supernatant exchanges performed with the cultures containing activated carbon in the present study represented dynamic substrate and hydraulic loading conditions.

All of the cultures in the activated carbon assay were agitated on a shaker table at 175 RPM. This mixing intensity was not sufficient to suspend or fluidize the activated carbon particles. However, the particles were in continuous motion, colliding with each other, on the bottom of the serum bottles.

A non-reactive, neutrally-buoyant dye was added to serum bottles containing 75 mg activated carbon in 12.5 mL distilled water and agitated at 175 RPM to observe the mixing characteristics. The circular motion of the shaker table produced spiral flow lines in the liquid from the dye suggesting that laminar flow conditions existed. The dye was completely dispersed after ~5 seconds indicating that some secondary mixing was occurring. This mixing was probably due to the motion of the activated carbon particles on the bottom of the serum bottle.

Hydraulic shear and abrasion are two mechanisms that would have promoted biomass loss in the activated carbon cultures. Sloughing may have been occurring immediately after exchanging 88% of the supernatant, if the replaced supernatant caused a significant change in the environmental conditions in the serum bottles.

The 12% supernatant cultures demonstrated substantially lower phenol removal capability than culture B (Figures 5.13a to 5.13d). The 12% supernatant cultures exhibited a decreasing trend in their rate of phenol removal with subsequent supernatant exchanges. This suggests that suspended biomass concentrations in the remaining 12% supernatant with the cultures containing activated carbon played a minor role in removing phenol compared to surface-attached biomass.

The total rates of phenol removal in the 12% supernatant cultures were the lower than in the 76% supernatant cultures. However, the removal rates per unit volume of inoculum (removed from the cultures containing activated carbon) were initially greater in the 12% supernatant cultures. Recall that the inoculum volume was 1.5 mL for the 12% cultures and 9.5 mL for the 76% cultures (section 5.4.1.1d). The specific phenol removal rate in the 12% supernatant culture was 28 mg phenol/mL inoculum/d (Figure 5.13a) and 11 mg/mL inoculum/d (Figure 5.13d). The specific removal rate in the 76% supernatant culture was 19 mg phenol/mL inoculum/d (Figure 5.13a) and 12 mg/mL inoculum/d (Figure 5.13d). Both of these specific rates were calculated from incubation day 1 to 3. The 76% cultures received $\frac{9.5}{1.5} = 6.3$ times the inoculum volume of the 12% cultures, however the 76% cultures did not possess 6.3 times the specific activity of the 12% cultures.

The 12% cultures were originally intended to provide a direct comparison to suspended biomass activity that remained in the supernatant after the exchange in the cultures containing activated carbon. The specific removal rate in culture B was 81 mg phenol/mL remaining/d (Figure 5.13a) and

75 mg/mL remaining/d (Figure 5.13d). These values clearly indicate that specific biomass activity associated with the activated carbon (i.e. culture B) was substantially higher than suspended biomass (i.e. the 12% supernatant cultures). This demonstrates the advantage of a biological support and the importance of biomass retention during startup of an anaerobic process with a short HRT.

The phenol removal rates of cultures A, B, C, and the 12% and 76% supernatant cultures were intended as a qualitative, not quantitative, measure of biomass activity. One uncontrollable variable in the bioassay was the supernatant exchange medium which contained 70% by volume autoclaved municipal digestor supernatant (section 5.4.1.1a and d). This mixture may have given the 12% cultures an advantage to degrade phenol compared with the 76% cultures. The 12% cultures received 10.5 mL of this mixture while the 76% culture only received 2.5 mL (section 5.4.1.1d). However, the cultures containing activated carbon received 10.5 mL of this mixture during the exchange and exhibited superior phenol removal rates compared to the 12% culture.

The adsorption rate of phenol to activated carbon in the presence of bacteria and after 11 repeated phenol loadings (Table 5.8), along with phenol adsorption to virgin activated carbon in the absence of bacteria, is shown in Figure 5.14. Moderate uptake of phenol occurred over the first 35 min in the serum bottle with bacteria. Aqueous phenol concentrations

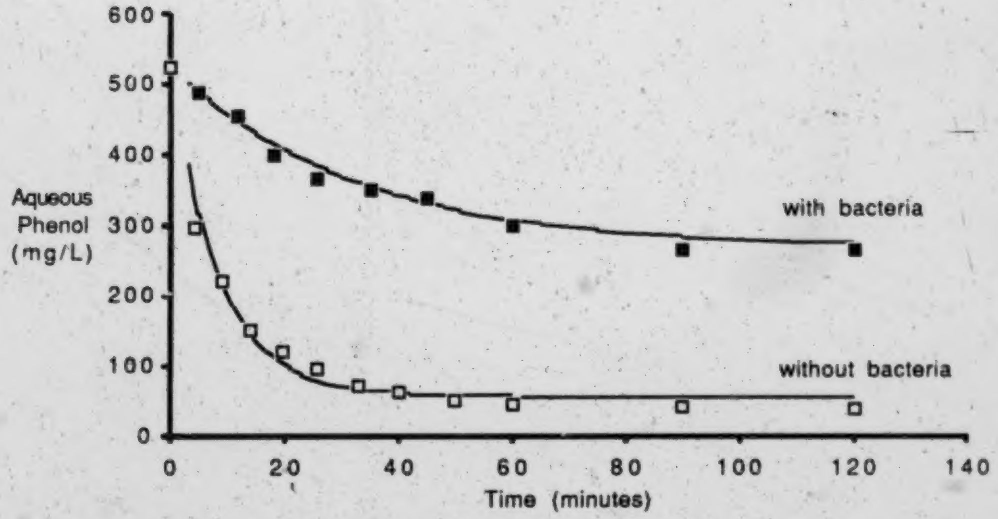


Figure 5.14 Phenol Adsorption on F300 Activated Carbon in the Presence of Biofilm Growth and Prior to the Addition of Bacteria

decreased from 520 to 350 mg/L during this time. Phenol levels were reduced from 350 to 265 mg/L over the next 85 min. The biological uptake of phenol over the 2 h period was not substantial (≈ 5 mg/L/h based on the average of 119 mg/L/d, Table 5.11). The aqueous phenol concentration after 60 min (322 mg/L) agrees well with the mean aqueous phenol concentration observed during similar phenol loading conditions (336 ± 22 mg/L, Table 5.10). This observation further supports the suggestion that bioregeneration was occurring under these conditions.

The phenol adsorption rate data in the presence of bacteria was modelled using equation (5-3) by assuming the adsorption rate was 1st order with respect to time:

$$C_{(t)} = [C_{(o)} + C^*]e^{-kWt/V} + C^*, \qquad \text{(Eqn. 5-3)}$$

A non-linear least squares method was used to fit the rate data to the model in equation (5-3):

$$C_{(t)} = 252e^{-t/32.062} + 268. \qquad \text{(Eqn. 5-13)}$$

Therefore:

$$k = \frac{0.0125\ \mathrm{L}}{32.062\ \mathrm{min}\ (0.075\ \mathrm{g})} = 0.00520\ \frac{\mathrm{L}}{\mathrm{g \cdot min}}, \qquad \text{(Eqn. 5-14)}$$

(95% confidence interval: $0.00431 \leq k \leq 0.00608$), and

$C^* = 268$ mg/L,

(95% confidence interval: $249 \leq C^* \leq 286$).

The quasi-equilibrium constant q^* is determined from:

$$q^* = [C_{(o)} - C^*]\frac{V}{W}. \qquad \text{(Eqn. 5-10)}$$

Substituting $C_{(o)}$ (520 mg/L), C^* (268 mg/L), W, and V into equation (5-12) gives:

$$q^* = [520 - 268]\,\frac{mg}{L}\cdot\frac{0.0125\ L}{0.075\ g} = 42.0\,\frac{mg}{g}, \qquad \text{(Eqn. 5-15)}$$

(95% confidence interval: $39.0 \le q^* \le 45.2$).

The apparent rate constant for phenol adsorption to virgin activated carbon in the absence of bacteria was determined previously:

$$k = 0.0203\,\frac{L}{g\cdot min}. \qquad \text{(Eqn. 5-5)}$$

Similarly, the quasi-equilibrium constant for phenol adsorption to virgin activated carbon in the absence of bacteria was determined previously:

$$q^* = 78.3\,\frac{mg}{g}. \qquad \text{(Eqn. 5-11)}$$

The apparent rate constants for phenol adsorption in the absence of bacteria (equation 5-5) and phenol adsorption in the presence of bacteria and after repeated phenol loadings (equation 5-14) can be compared by dividing one by the other:

$$\frac{k_{w/o\ bacteria}}{k_{w/bacteria}} = 0.0203/0.00520 = 3.9. \qquad \text{(Eqn. 5-16)}$$

Similarly, the quasi-equilibrium constants for phenol adsorption in the absence of bacteria (equation 5-11) and phenol adsorption in the presence of bacteria (equation 5-15) can be compared:

$$\frac{q^{*}_{w/o\ bacteria}}{q^{*}_{w/bacteria}} = 78.3/42.0 = 1.9. \qquad \text{(Eqn. 5-17)}$$

Equation (5-16) demonstrates the decrease in the apparent adsorption rate. This decrease suggests a reduction in the mass flux of phenol in the pores. The mass flux of phenol in activated carbon pores can be described:

$$F = -DA[\frac{dC}{dx}], \text{ where:} \qquad \text{(Eqn. 5-18)}$$

F = mass phenol flux (mg/h),

D = diffusion coefficient (cm^2/h),

A = pore cross-sectional area (cm^2), and

$\frac{dC}{dx}$ = phenol concentration gradient ($\frac{mg}{cm^3 \cdot cm}$).

Equation (5-18) shows that the mass phenol flux (F) depends on the diffusion coefficient *(D)*, the pore cross-sectional area (A), and the concentration driving force in the pores ($\frac{dC}{dx}$).

All three parameters are likely responsible for the reduced mass phenol flux. A lower combined diffusion coefficient *(D_n)* corresponding to the net diffusion of phenol through the glycocalyx and cellular mass (biomass), and water would contribute to a decrease in the mass flux.

The presence of biomass in the activated carbon pores may have reduced the effective cross-sectional area of the pores thereby restricting (or blocking) the access of phenol. Faust and Aly (1983) reported the area/molecule of phenol as 52 $A^{\circ 2}$ $(5.2 \cdot 10^{-7}\ \mu m^2)$. Assuming a spherical shape for phenol gives it a diameter of $\approx 8 \cdot 10^{-4}$ µm. The average diameters of single-celled bacteria are 0.3-2 µm (Nester et al., 1983). A phenol molecule is ≈375-3,750 times smaller than average sized bacteria. The accumulation of bacteria in the macropores of activated carbon may interfere with phenol traversing the pores, however the phenol molecules are small enough to pass through the biomass growth to subsequent adsorption sites.

If the same phenol adsorption capacity was available on activated carbon in the presence and absence of biomass growth, the concentration driving force $(\frac{dC}{dx})$ should be the same for each situation. Any reduction in the mass phenol flux would then be due to a reduction in the diffusion coefficient and/or the pore cross-section area. However, considering that the cultures containing activated carbon were previously loaded with phenol 11 times (Table 5.8), it is likely that a reduced concentration driving force was partially responsible for a decrease in the mass phenol flux in activated carbon pores containing attached-biomass.

Equation (5-17) demonstrates the apparent reduction in the phenol adsorption capacity (measured over a 2 h period). The presence of biomass in the activated carbon pores and/or a reduction of adsorption sites (taken up by previously adsorbed phenol) are likely responsible for the observed reduction. It may be that the presence of biomass restricted the access of phenol in the pores giving the net effect or appearance of a reduction in the adsorption capacity (measured over the 2 h period).

The apparent reduction in phenol adsorption capacity observed between incubation day -5 and 7 for cultures A, B, and C (Figure 5.11) can be compared to the apparent reduction observed in culture B between incubation day 0 and 91 (Figure 5.14 or equation 5-17).

The discussion presented at the beginning of section 5.4.1.2 mentions that virgin activated carbon in cultures A, B, and C adsorbed 9.3 mg of a 12.2 mg phenol slug dose (on incubation day -5). This corresponds to a quasi-equilibrium adsorption capacity of (based on a 1 h contact period):

$$q^* = \frac{9.3 \text{ mg phenol}}{0.075 \text{ g activated carbon}} = 124 \frac{\text{mg}}{\text{g}}. \qquad \text{(Eqn. 5-19)}$$

The next addition of 12.2 mg phenol to these cultures on incubation day 7 saw only 3.9 mg adsorbed corresponding to a quasi-equilibrium adsorption capacity of (based on a 1 h contact period):

$$q^* = \frac{3.9 \text{ mg phenol}}{0.075 \text{ g activated carbon}} = 52 \frac{\text{mg}}{\text{g}}. \qquad \text{(Eqn. 5-20)}$$

The apparent reduction of q^* observed over the 12 day period in cultures A, B, and C (incubation day -5 to 7) = 124/52 = 2.4.

This observation was based on measurements made after a 1 h contact period. The reduction in q^* observed in culture B over the 91 day incubation period (equation 5-17) was based on measurements made after a 2 h contact period. However, Figure 5.14 (culture B) shows that the amount of phenol adsorbed to activated carbon in the presence and absence of biomass is similar after 1 and 2 h. From this discussion it becomes apparent that the reduction in q^* in cultures A, B, and C after incubation day 7 (Figure 5.11), is more likely due to an actual reduction in adsorption sites. It is unlikely that biomass had accumulated in the activated carbon pores in these cultures (after 7 days incubation) to the same degree as what would be expected in culture B (after 91 days incubation).

5.4.2 Synthetic Ion Exchange Resins

5.4.2.1 Procedures

a) The ion exchange resins were prepared for the biological assay in the following manner.

The Dowex™ MSC-1 cation exchange resin (average size 300-850 µm, Dow Chemical Co.) was loaded with calcium (Ca^{2+}) for the assay. 100 mL of the wet resin (initially loaded with Na^+) was added to 250 mL solution of 11.1% $CaCl_2$ (111 g/L) and equilibrated on a shaker table (150 RPM) at room temperature for 65 h. The solution was drained from the beaker and the resin was washed with 4 volumes (250 mL) of deionized distilled (DD) water. The resin was transferred to a vacuum filtration flask and filtered with 2 L of DD water.

The Ambergard™ XE-352 anion exchange resin (average size 300-850 µm, Rohm and Haas Co.) was already loaded with Cl⁻. 100 mL of this wet resin was washed with 4 volumes (250 mL) of deionized distilled (DD) water and filtered with 2 L of DD water (by vacuum filtration). Both resins were used immediately after preparation for the assay.

b) The bioassay was performed in 58 mL volume serum bottles. Six bottles (designated D, E, F for MSC-1 cationic resin; and G, H, I for XE-352 anionic resin) were prepared according to the procedure described in section 3.1.1 with one exception. After flushing the bottles and prior to the addition of the liquid contents, the resins were weighed out and added to the serum bottles according to the procedure described in part c) below. The bottles then received 10.9 mL of the growth medium mixture (see section 5.3.1.1). The bottles were capped, sealed, and given 0.1 mL of a sulfide solution (see Table 3.1) and 6.1 mg phenol (i.e. 0.5 mL of a phenol stock solution 12,200 mg/L). The bottles were agitated on a shaker table (175 RPM) in an incubator (37°C) for 2 days. A 1 mL suspension of acclimated phenol-degrading consortium was added to each bottle (12.5 total liquid volume). The removal of phenol and biological methane production were monitored.

c) The resins were weighed out and added to the serum bottles according to the method of van Vliet et al. (1980). The wet resins were placed in 15 mL coarse sintered glass funnels, which fitted snugly into metal support sleeves. The funnels were capped, and the complete assembly centrifuged at 4,000 RPM for 2 min. The funnel cover prevented drying of the upper layers of the resins via air turbulence during centrifugation. The resins were then weighed out and transferred to the serum bottles. At the time of weighing, an additional set of the resin samples were weighed into tared vials, dried to a constant weight at 110°C to determine the moisture content and % dry resin. An average dosage of 88.6 mg of dry MSC-1 resin was added to serum bottles D, E, and F. An average dosage of 25.6 mg of dry XE-352 resin was added to serum bottles G, H, and I.

d) The cultures were allowed to remove phenol to concentrations less than 50 mg/L. At this point the cultures were either supplemented with 6.1 mg phenol from a reduced stock solution, or, they had 88% of the liquid contents removed and replaced with a bacteria-free medium prior to phenol supplementation. The supernatant exchange procedure, and the setup of new cultures using the removed supernatant as innoculum were the same as described in section 5.4.1.1.

e) The three cultures (D, E, and F) containing MSC-1 cation exchange resin were fed 6.1 mg phenol on incubation day 11 and day 17. On day 21, these cultures had 88% of their supernatant removed and replaced, and were again fed 6.1 mg phenol. The cultures were monitored for another 12 days before being abandoned.

The schedule of operations performed on the three cultures (G, H, and I) containing XE-352 anion exchange resin are summarized in Table 5.12 The cultures were maintained for approximately 95 days.

f) The short term phenol adsorption kinetics were investigated in culture H (containing XE-352 resin) when it was supplemented with phenol on day 48. The bottle was given 6.1 mg phenol and analysis were immediately performed (section 3.2.6). Aqueous phenol concentations were analyzed over a period of 2 h. The bottle was continuously shaken (175 RPM) at 37°C during this time.

5.4.2.2 **Results and Discussion**

MSC-1 Cation Exchange Resin

The behavior of cultures D, E, and F with respect to phenol removal is displayed in Figure 5.15. All three cultures exhibited similar behavior throughout the bioassay. The bottles were initially given 6.1 mg phenol on day -2 and allowed to equilibrate. The cultures were inoculated with bacteria on day 0 and supplemented with 6.1 mg phenol on day 11 and day 17. Just prior to phenol supplementation on day 21, the cultures

Table 5.12 Operation Schedule for XE-352 Resin Bioassay

Incubation Day	Operation Performed
-2	Serum bottle setup, fed 0.5 mL phenol
0	Bottles inoculated with bacteria
6	Fed 0.5 mL phenol
11	Fed 0.5 mL phenol
16	1st S.E., fed 0.5 mL phenol, 1st S.N.C.

Cultures operated individually after day 23:

G	H	I	
26	25	24	2nd S.E., fed 0.5 mL phenol, 2nd S.N.C.
38	32	31	3rd S.E., fed 0.5 mL phenol, 3rd S.N.C.
47	38	38	4th S.E., fed 0.5 mL phenol, 4th S.N.C.
54	43	43	5th S.E., fed 0.5 mL phenol, 5th S.N.C.
59	48	48	6th S.E., fed 0.5 mL phenol, 6th S.N.C.
64	52	52	7th S.E., fed 0.5 mL phenol
68	57	56	8th S.E., fed 0.5 mL phenol, 7th S.N.C.
-	61	60	9th S.E., fed 0.5 mL phenol
-	64	63	10th S.E., fed 0.5 mL phenol, 8th S.N.C.
-	67	66	11th S.E., fed 0.5 mL phenol
-	70	69	12th S.E., fed 0.8 mL phenol

Cultures were not monitored from day 71-76.

G	H	I	
-	76	76	13th S.E., fed 0.8 mL phenol
-	-	80	14th S.E., fed 1.1 mL phenol, 9th S.N.C.

phenol - Reduced phenol stock solution (12,200 mg/L).

S.E. - Supernatant exchange procedure whereby 88% (11 mL) of the ion exchange resin culture supernatant was removed and replaced with reduced, bacteria-free growth medium.

S.N.C. - Setup of new cultures using supernatant (removed from resin cultures) as inoculum.

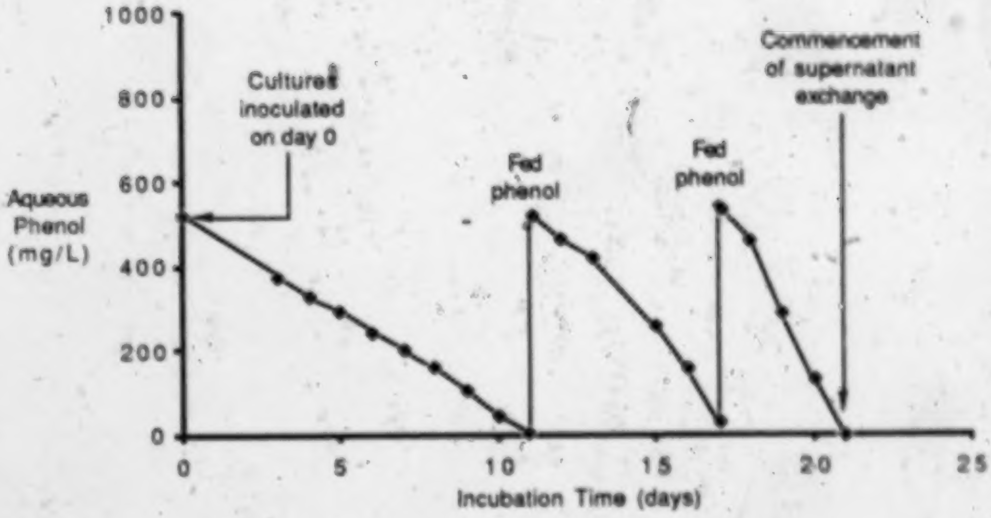


Figure 5.15 Aqueous Phenol Behavior of Anaerobic Cultures Containing 7,090 mg/L MSC-1 Resin

had 88% of the supernatant removed and replaced with biomass-free liquid.

The rate of phenol removal increased after each feed episode in Figure 5.15 (47 mg/L•d from day 0 to 11, 100 mg/L•d from day 11 to 17, and 164 mg/L•d from day 17 to 21). The cumulative methane production for the same time period is shown in Figure 5.16. Complete conversion of phenol to methane was observed up to day 21 (corresponding to the first and only supernatant episode) because no adsorption of phenol to the MSC-1 resin occurred. This is in contrast to observed methane production from the cultures containing activated carbon (Figure 5.12). Complete conversion of phenol to methane was not observed in the activated carbon cultures because a fraction of the added phenol was adsorbed to the activated carbon and unavailable to bacteria.

Figure 5.16 also shows that the observed methane production deviated from the expected production at the first supernatant exchange. This problem was inherent to the exchange procedure. The culture headspace was always pressurized with biological gas to ensure a positive pressure during the exchange procedure. Consequently, some gas was lost during each exchange. This problem was not as obvious in the activated carbon cultures because phenol adsorption and bioregeneration tended to mask the effect.

The removal of phenol or metabolites of phenol biodegradation (e.g. acetate) in the supernatant during the exchange also accounted for a reduction in the observed

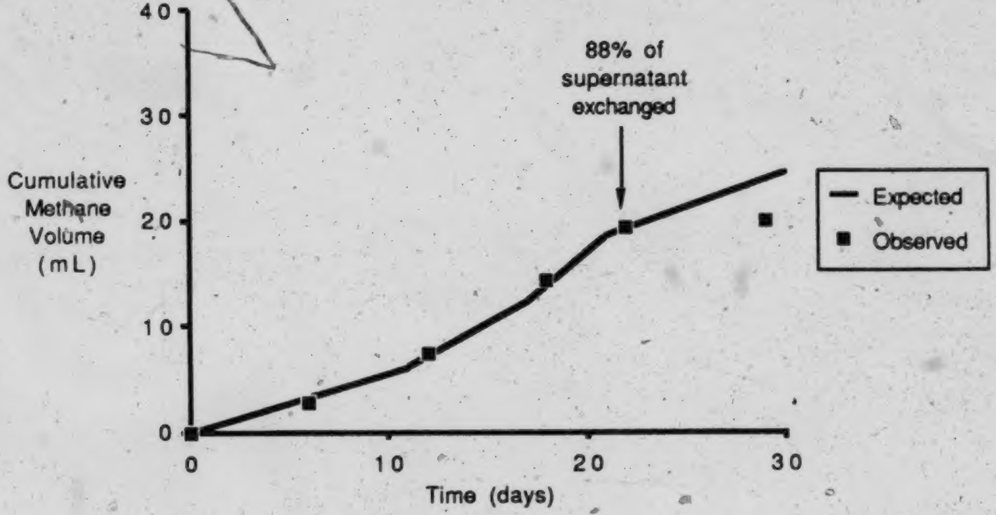


Figure 5.16 Expected and Observed Methane Production of Anaerobic Cultures Containing 7,090 mg/L MSC-1 Resin

methane production in cultures D, E, and F. The total quantity of methane was measured in the 76% supernatant culture derived from culture E after the first supernatant exchange and found to be 7.8 mL. The expected methane production in the 76% supernatant culture was 5.8 mL (based on the addition of 6.1 mg phenol). The excess methane volume observed 7.8 - 5.8 = 2 mL was likely from biological utilization of phenol or phenol metabolites that were transferred in the supernatant from culture E during the exchange.

The behavior of aqueous phenol in cultures D, E, and F after the first supernatant exchange is shown in Figures 5.17a to 5.17c. The 76% supernatant culture from D was contaminated with oxygen during setup, consequently, no biological activity was observed in this culture (Figure 5.17a). The 76% supernatant cultures from E and F had the highest phenol removal rates. Both cultures removed phenol at a rate of 169 mg/L•d. The removal rate in cultures E and F prior to the exchange (164 mg/L•d from day 17 to 21) is comparable to the rate observed in their 76% supernatant after the exchange. This suggests that the supernatant bioactivity continued to increase after the exchange.

The 12% supernatant cultures removed phenol at rate of 34 to 36 mg/L•d, while the cultures containing MSC-1 resin only removed phenol at a rate of 17 to 21 mg/L•d (Figures 5.17 a to c). This suggests that the MSC-1 resin may have impaired phenol removal, or, that distribution of suspended biomass

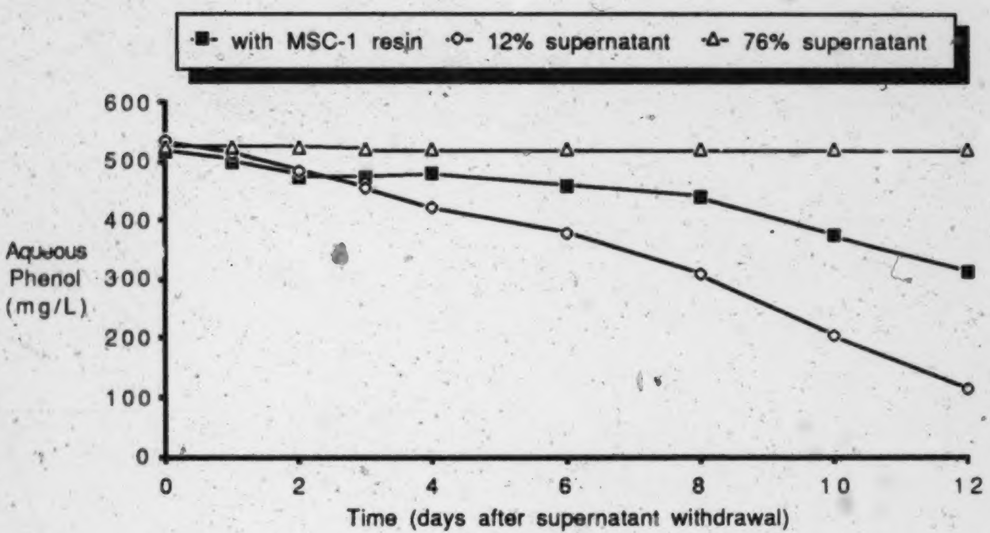


Figure 5.17a Phenol Biodegradation in Culture D with MSC-1 Resin and in Supernatant Removed from Culture D

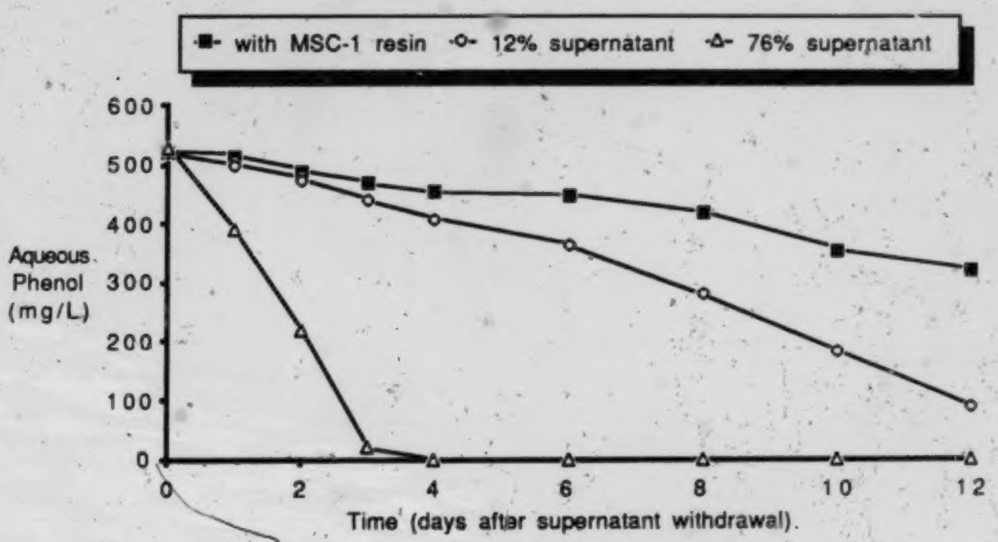


Figure 5.17b Phenol Biodegradation in Culture E with MSC-1 Resin and in Supernatant Removed from Culture E

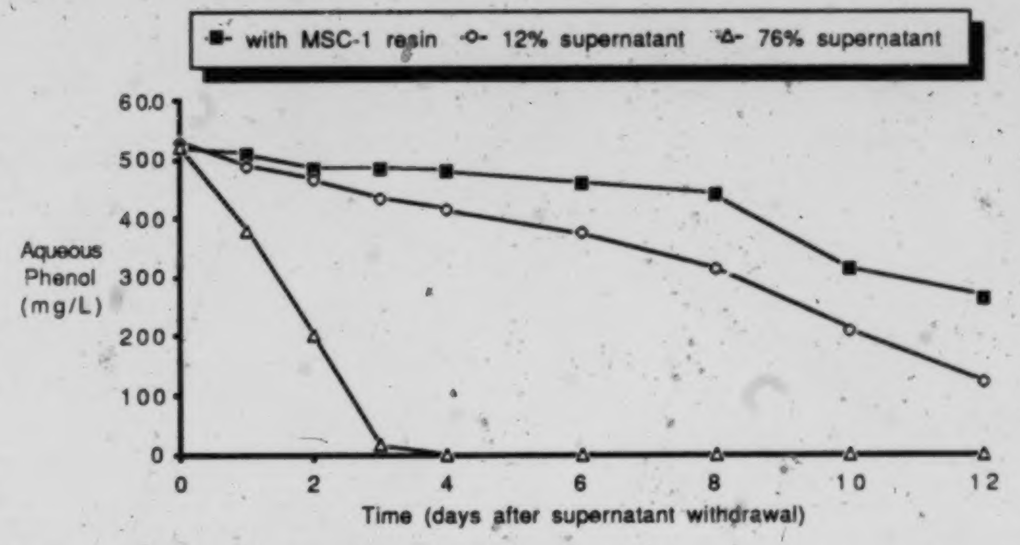


Figure 5.17c Phenol Biodegradation in Culture F with MSC-1 Resin and in Supernatant Removed from Culture F

between the three cultures (88%, 12%, and the resin culture) was not uniform during the exchange.

Exchanging 88% of the supernatant in the cultures containing MSC-1 resin reduced the phenol removal rate from 164 mg/L•d (day 17 to 21) to 17 to 21 mg/L•d (after day 21) - a factor of ≈9 times (≈88% reduction). This observation, and the fact that both the 12% and 76% supernatant cultures had higher bioactivities suggests little or no biomass activity was associated with the MSC-1 resin. The pore volume and surface area properties of MSC-1 resin (Table VII.3, Appendix VII) suggest that this resin would not be favorable for biofilm development. Figure 5.5 indicates that this resin has negligible internal pore volume to shelter bacteria (i.e. 0.007 mL/g associated with mean pore diameters ≥15.13 µm).

The results from this bioassay demonstrated the importance of removing the supernatant in order to study the bioactivity associated with attached-growth bacteria. Removing 88% of the supernatant reduced the phenol removal rate by ≈88% (164 to 17-21 mg/L•d).

XE-352 Anion Exchange Resin

The behavior of cultures G, H, and I with respect to phenol removal is displayed in Figure 5.18. All three cultures exhibited similar behavior after 16 days incubation. The bottles (minus bacteria) were initially given 6.1 mg phenol on day -2 and allowed to equilibrate. The cultures were inoculated with bacteria on day 0 and supplemented with 6.1

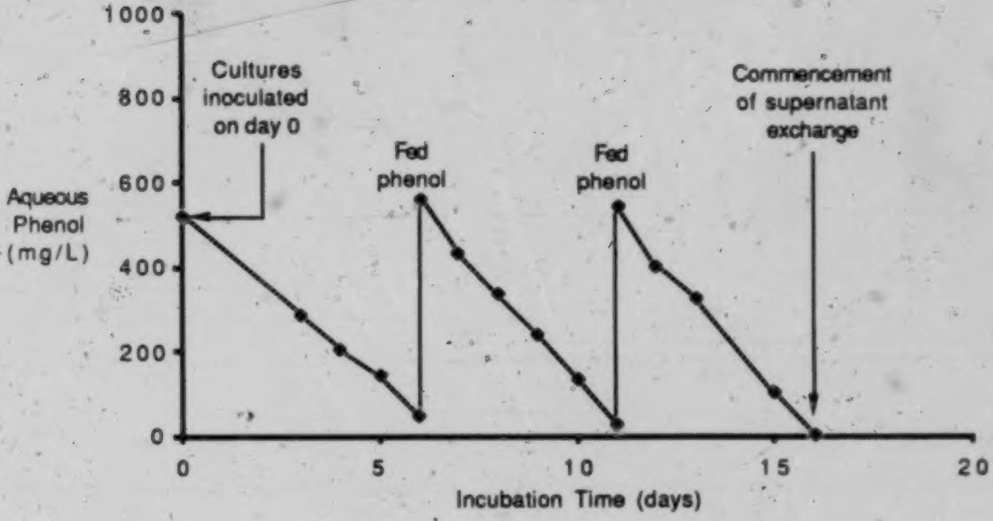


Figure 5.18 Aqueous Phenol Behavior of Anaerobic Cultures Containing 2,050 mg/L XE-352 Resin

mg phenol on day 6 and day 11. The phenol removal rate increased from 75 mg/L·d (day 0 to 6) to 110 mg/L·d between day 11 to 16. Just prior to phenol supplementation on day 16, the cultures had 88% of the supernatant removed and replaced with biomass-free liquid.

The cumulative methane production for the same time period is shown in Figure 5.19. Complete conversion of phenol to methane was observed up to day 12. The observed methane production deviated from the expected production after supernatant exchanges began. Figure 5.19 demonstrates the cumulative loss of methane with consecutive volume exchanges.

The behavior of culture I and the cultures containing 12 and 76% of the supernatant from culture I during the 5th, 6th, 8th, and 10th consecutive supernatant exchange is shown in Figures 5.20a to 5.20d. The behavior of culture I during the first four supernatant exchanges is shown in Figures X.3a to X.3d (Appendix X). The behavior of cultures G and H from incubation day 25 to 76 are shown in Figures X.1a to X.1g (G) and Figures X.2a to X.2h (H) in Appendix X.

The 12% supernatant cultures exhibited decreasing ability to remove phenol with successive supernatant withdrawals for G, H, and I. The phenol removal capability for these cultures decreased to the point where little or no removal was observed after four withdrawals (Figure X.1d for culture G, Figure X.2d for H, and Figure X.3d for I). Some of the subsequent withdrawals did not test the 12% supernatant as a result. A

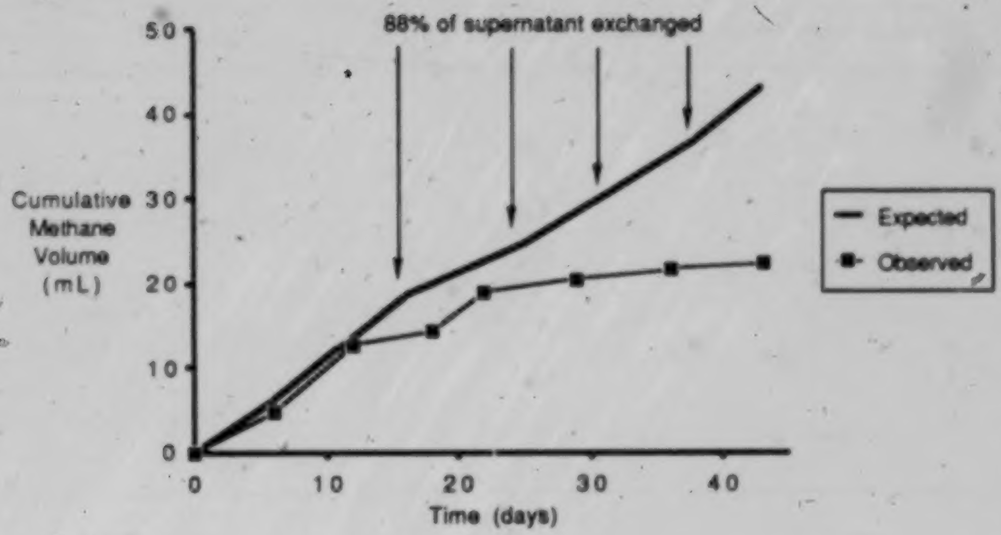


Figure 5.19 Expected and Observed Methane Production of Anaerobic Cultures Containing 2,050 mg/L XE-352 Resin

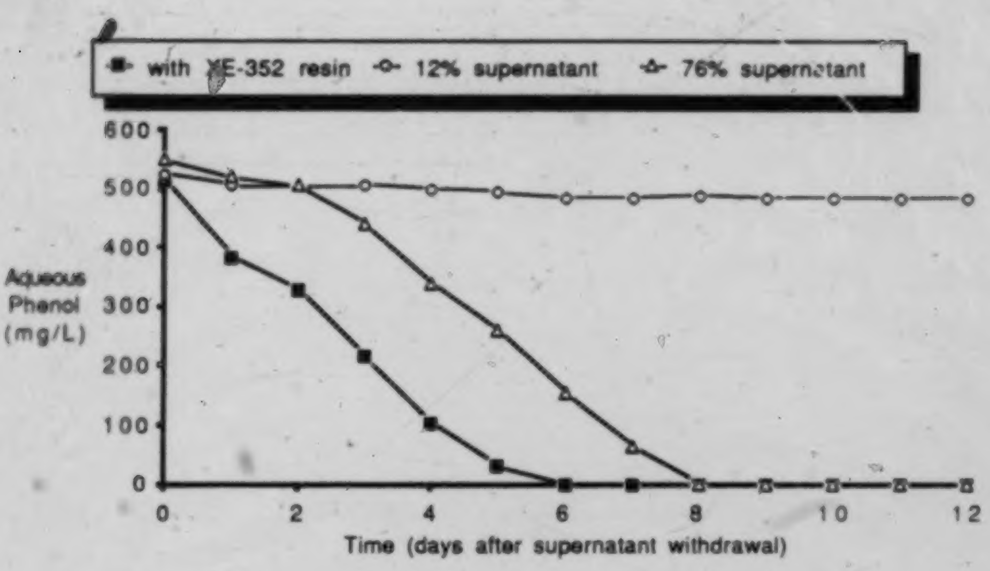


Figure 5.20a Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (5th consecutive supernatant withdrawal)

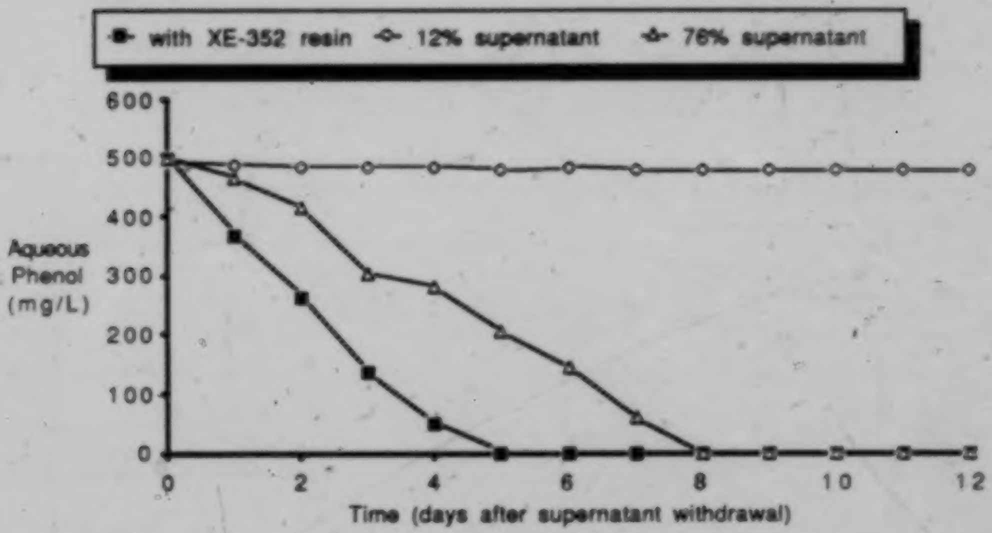


Figure 5.20b Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (6th consecutive supernatant withdrawal)

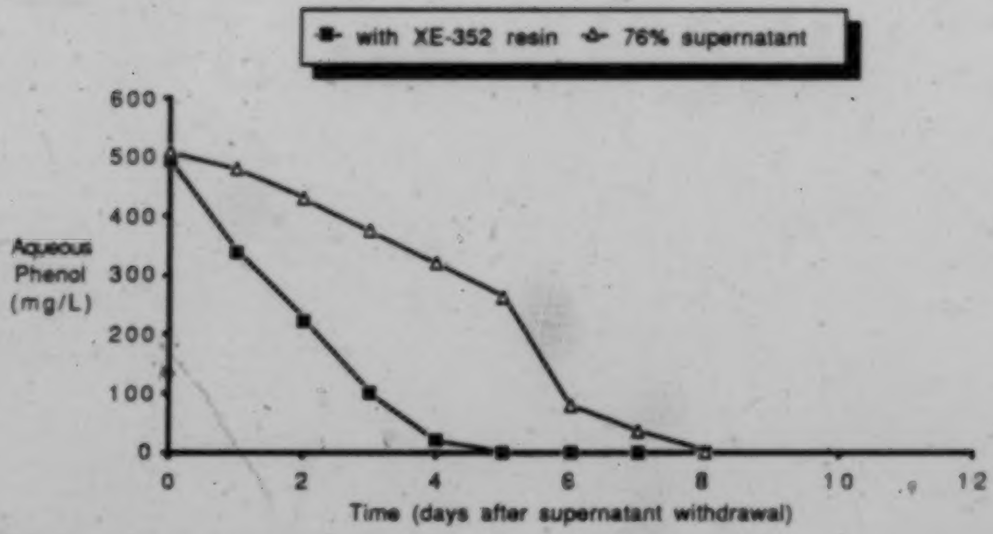


Figure 5.20c Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (8th consecutive supernatant withdrawal)

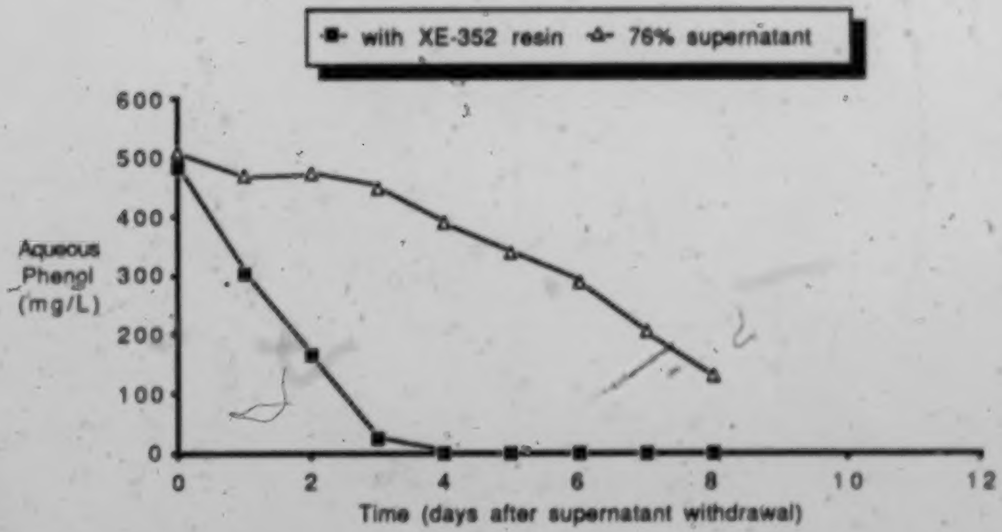


Figure 5.20d Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (10th consecutive supernatant withdrawal)

possible explanation for the minimal bioactivity observed in the 12% supernatant cultures can be seen in Figures 5.20a and 5.20d with the 76% supernatant cultures. A lag period occurred for 2 to 3 days after the 76% cultures were setup. Phenol removal increased to a near steady-state rate after the 2 to 3 day lag. This phenomenon was again observed in many of the 76% supernatant cultures shown in Appendix X, and was probably due to oxygen contamination during setup. This would have occurred in the 12% supernatant cultures also. The 76% cultures contained 6.3 times the inoculum volume as the 12% cultures and, therefore, contained approximately 6.3 times the bioactivity as the 12% cultures. The anaerobic bacteria were able to recover in the 76% cultures but not in the 12% cultures after a short lag. The phenol removal rate clearly demonstrates the observed lag in the 76% cultures: 23 mg/L•d from day 0 to 2 and 88 mg/L•d from day 2 to 8 (Figure 5.20a), 20 mg/L•d from day 0 to 3 and 64 mg/L•d from day 3 to 8 (Figure 5.20d).

The XE-352 resin-containing cultures G and H demonstrated similar behavior to culture I. Figures 5.20a to 5.20d show that the XE-352 resin-containing culture I consistently had a greater capability for phenol removal. The estimated phenol biodegradation rates between the 1st and 3rd day after each supernatant withdrawal in cultures G, H, and I are shown in Figure 5.21 for all the supernatant exchanges performed. A definite pattern is observed in Figure 5.21. The phenol removal rate in the ~~XE-352 resin~~ cultures increased

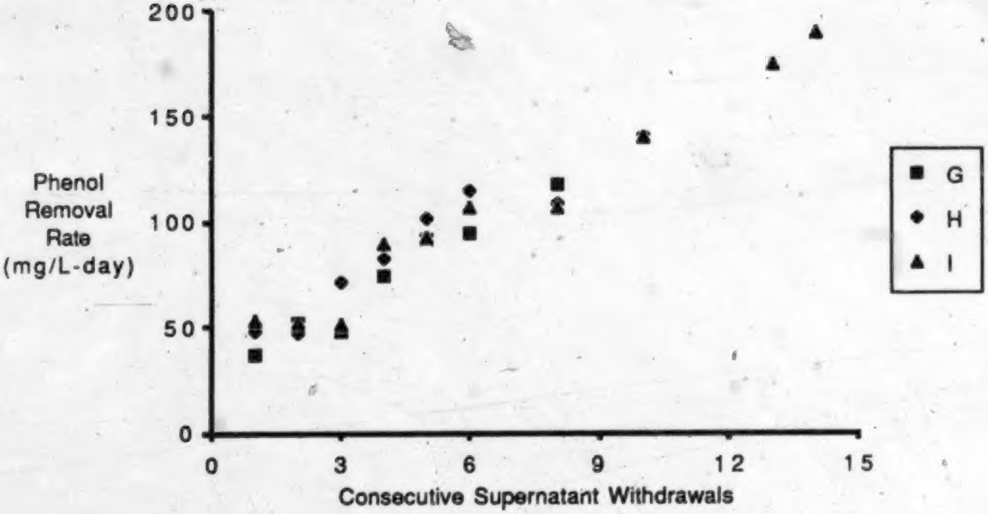


Figure 5.21 Phenol Removal Rate in Cultures G, H, and I with XE-352 Resin at Each Supernatant Exchange

steadily throughout the assay (up to 190 mg/L·d at incubation day 88). This suggests the developing resin-attached biomass had not yet reached its full potential after 88 days incubation (14th supernatant exchange - culture I).

This is in contrast to what was observed in the activated carbon cultures (Table 5.8 and 5.11). The activated carbon cultures A, B, and C had an average phenol removal rate of 119 ± 9 mg/L·d. These cultures did not show any increase in phenol removal rates during the four consecutive supernatant exchanges (i.e. after 75 days incubation).

The phenol removal rates for the 76% supernatant cultures derived from XE-352 resin-containing cultures are shown in Table 5.13. The cultures exhibited similar removal rates throughout the whole assay, except from the 1st supernatant exchange. The phenol removal rates from the XE-352 resin 76% supernatant cultures (64 ± 15 mg/L·d) were substantially lower than what was observed for the activated carbon 76% supernatant cultures (150 ± 39 mg/L·d). The removal rate for the activated carbon 76% cultures was averaged over incubation day 75 to 100, whereas the rate from the XE-352 resin 76% cultures was determined from incubation day 16 to 88. The comparison is valid because the removal rates from each set of cultures did not change substantially over the period the rates were measured.

The behavior of XE-352 resin-containing cultures from the 13th and 14th supernatant withdrawals is shown in Figure

Table 5.13 Phenol Removal Rates in 76% Supernatant Cultures from XE-352 Resin Bioassay

Consecutive Supernatant Exchange	Phenol Removal Rate (mg/L·d)		
	G	H	I
1st	131	188	115
2nd	72	48	44
3rd	45	73	69
4th	41	80	88
5th	39	79	76
6th	65	62	68
8th	68	76	80
10th	-	44	64
14th	-	-	81

Mean = 64
S.D. = 15

Note: S.D. = standard deviation.
Removal rates from the 1st supernatant exchange were not used used to calculate the mean and S.D.

5.22 and Figure 5.23 respectively. Cultures H and I were fed 9.6 mg phenol after their 12th supernatant withdrawal, and again after their 13th supernatant withdrawal. This mass of phenol was equivalent to 770 mg/L in the cultures. The phenol removal rate after the 13th supernatant withdrawal in culture H (100 mg/L•d) was substantially lower than the rate from culture I (175 mg/L•d) (Figure 5.22). Culture H was starved of phenol from day 75 to 76 (just prior to the 13th exchange) and had a lower capacity for phenol removal after the exchange. This short period of non-feeding in culture H resulted in a loss of bioactivity that was not temporary, suggesting a reduction in the quantity of active biomass. Culture I was fed 9.6 mg phenol when the aqueous phenol concentration was ≤50 mg/L (i.e. on demand) during this time and exhibited higher phenol removal capacity than culture H.

Culture I was given 13.1 mg phenol after the 14th supernatant exchange, and a culture with 76% of the supernatant removed from I was given 10.9 mg phenol (Figure 5.23). Culture I was not inhibited by the phenol spike concentration of 1,050 mg/L, and removed phenol at the highest rate observed yet (190 mg/L•d).

A distinct lag was evident with the 76% supernatant culture. This culture was only given a phenol spike concentration of 860 mg/L. Very little phenol removal was observed during the first few days of incubation (<15 mg/L•d). Phenol removal increased to 35 mg/L•d by day 5 and 83 mg/L•d

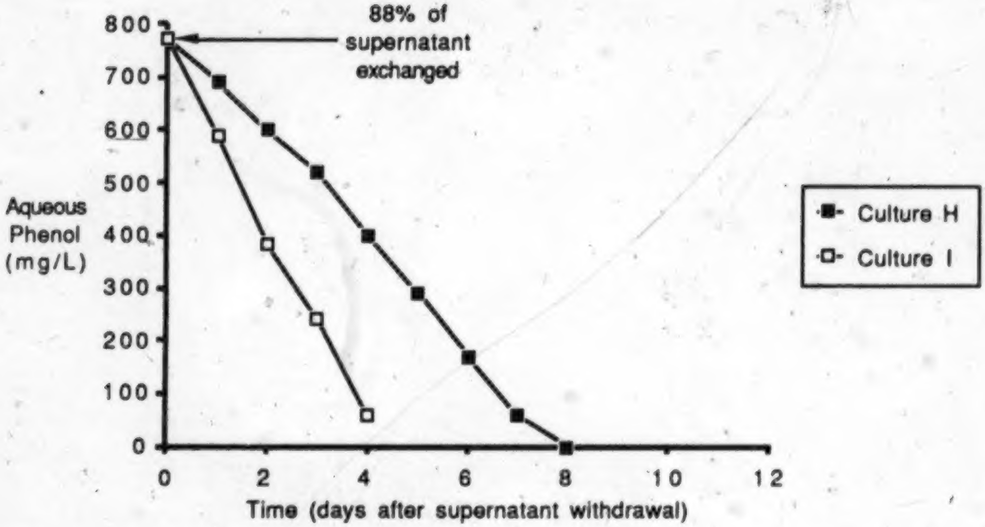


Figure 5.22 Phenol Biodegradation in Cultures H and I with XE-352 Resin (13th consecutive supernatant withdrawal)

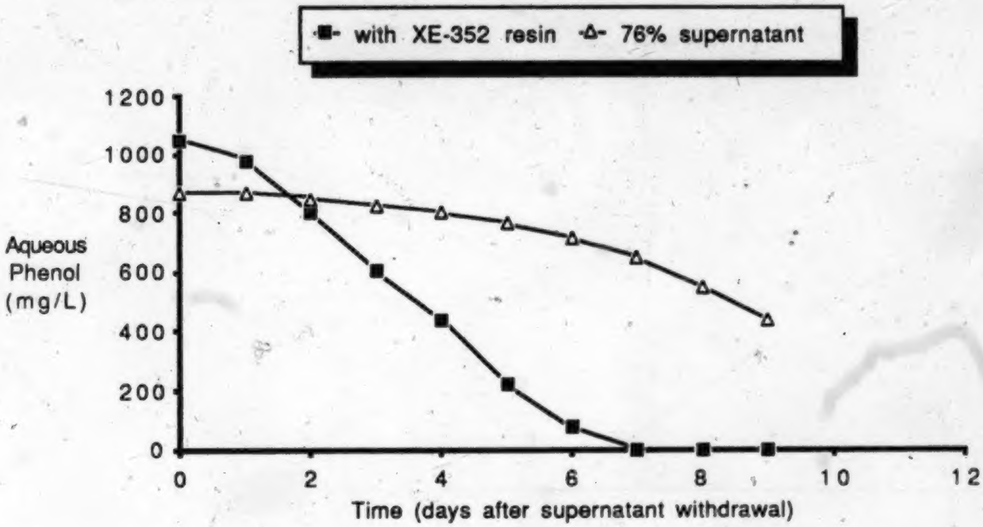


Figure 5.23 Phenol Biodegradation in Culture I with XE-352 Resin (14th consecutive supernatant withdrawal)

by day 8. This rate is comparable to previous 76% supernatant cultures (64 ± 15 mg/L·d) (Table 5.13).

The lack of inhibition observed in culture I, compared with the 76% supernatant culture, suggests the attached-growth organisms in culture I were buffered from the phenol spike concentration. The resin surface and/or internal pores may have offered attached bacteria protection from the high bulk liquid phenol concentration, and, therefore minimized the inhibition that was evident in the 76% supernatant culture containing suspended biomass.

The short term behavior of aqueous phenol concentration was observed in culture H after the 6th supernatant exchange (day 48). Culture H was given 6.1 mg phenol and the supernatant concentration was monitored over the next 2 h (Figure 5.24). The previous bioactivity in culture H (i.e. before day 48) would only account for the removal of 8 to 10 mg phenol over the 2 h period. Figure 5.24 shows the phenol concentration decreased from 490 to 450 mg/L within 30 min. The remainder of phenol unaccounted for in solution must have been associated with the resin-attached bacteria, or the resin itself. Boyd (1982) found that phenol adsorbed to hydrogen-bonding sites on organic or inorganic matter (clay) in soils. The mechanism of phenol adsorption was presumed to be H-bond formation between the phenolic hydroxyl and the bonding site on the soil surface. This mechanism may have accounted for the short term uptake of phenol from solution. The amount

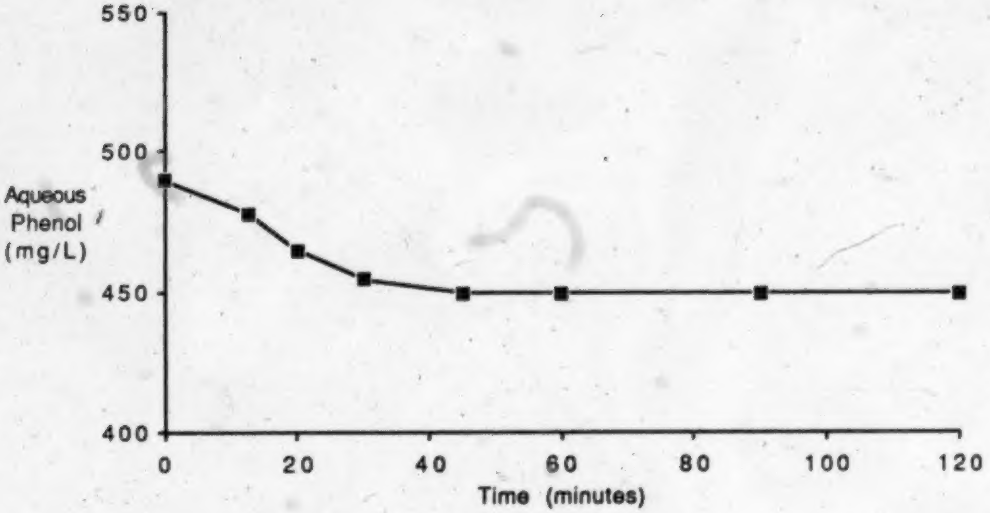


Figure 5.24 Short-Term Kinetic Removal of Phenol in Culture H with XE-352 Resin on Incubation Day 48

of phenol that would adsorb to the XE-352 synthetic resin in the absence of biomass was not investigated.

5.4.3 Comparison of Biological Supports

The revelant characteristics of the bioassays with different support materials are shown in Table 5.14. The bioassay containing MSC-1 resin had only one supernatant exchange performed from which the hydraulic residence time (HRT) for these cultures was estimated. The F300 activated carbon cultures (HRT = 5.8 days) and XE-352 resin cultures (HRT = 5.5 days) had similar average HRT during their period of study.

The bioactivies of anaerobic cultures containing the different support materials are summarized in Table 5.15. The rates of phenol removal (mg/d or g/d) from the cultures are compared:

1) per unit liquid volume (L),
2) per unit pore volume (mL) of the individual support materials (associated with mean pore diameters ≥15 µm, based on mercury porosimetry measurements),
3) per unit surface area (m^2) of the individual support materials (associated with mean pore diameters ≥15 µm, based on mercury porosimetry measurements),
4) per unit mass (g) of dry support material, and
5) per unit spherical surface area (m^2) of the individual support materials (assuming spherical particles of average mean diameter).

The phenol removal rate per unit pore volume and per unit surface area in the MSC-1 resin-containing cultures were not

Table 5.14 Characteristics of Support Material Bioassays

	Support Materials		
Characteristic	F300	MSC-1	XE-352
Support Material Dosage (mg/L)	6,000	7,090	2,050
Pore Volume (mL/g)[1]	0.192	0.007	1.220
Pore Volume $\left(\frac{\text{mL}}{\text{bottle}}\right)$[1]	0.0144	0.0006	0.0312
Surface Area (m^2/g)[1]	0.0173	0.0006	0.2270
Surface Area $\left(\frac{\text{m}^2}{\text{bottle}}\right)$[1]	0.0013	0.00005	0.0058
Spherical Area $\left(\frac{\text{m}^2}{\text{bottle}}\right)$[2]	0.000195	0.000219	0.000785
Period of Study[3]	d. 75-100	d. 21-44	d. 16-88
HRT (days)[4]	5.8	26.1	5.5

[1] Associated with mean pore diameters ≥15 µm based on mercury porosimetry measurements.

[2] Estimated spherical surface area assuming spherical particles.

[3] Days of incubation over which the bioactivity of the cultures containing support materials were closely observed.

[4] Estimated hydraulic retention time during the period of study based on the frequency of consecutive supernatant withdrawals.

Table 5.15 Comparison of Phenol Removal Rates During Support Material Bioassays

Parameter	Phenol Removal Rate	Support Material F300	Support Material MSC-1	Support Material XE-352
per L of liquid volume	$\frac{\text{mg phenol}}{\text{L} \cdot \text{day}}$	119	17-21	up to 190
per mL of pore volume	$\frac{\text{mg phenol}}{\text{mL pore volume} \cdot \text{day}}$	103	N.D.	76
per m^2 of surface area	$\frac{\text{mg phenol}}{\text{m}^2 \text{ surface area} \cdot \text{day}}$	1,140	N.D.	410
per gram of dry material	$\frac{\text{mg phenol}}{\text{g material} \cdot \text{day}}$	20	2.7	93
per m^2 of spherical area	$\frac{\text{g phenol}}{\text{m}^2 \text{ spherical area} \cdot \text{day}}$	7.6	0.8	10.9

Note: Pore volume and surface area of support materials are associated with mean pore diameters ≥15 µm as determined by mercury porosimetry.
N.D. = Not determined.

determined. These cultures removed phenol at a slower rate than the 12% supernatant cultures (Figure 5.17a to 5.17c). It was not possible to distinguish whether suspended or attached biomass was more important in removing phenol in the resin-containing cultures. Also, the phenol removal rate was reduced by 88% after 88% of the supernatant was exchanged. These observations strongly indicated that little or no bioactivity was associated with the bacteria attached to the resin. Calculation of phenol removal rate data from the MSC-1 resin-containing cultures would not be a valid representation of the activity of attached bacteria. Other phenol removal rates for this resin are reported for comparison purposes only. These rates clearly do not represent resin-associated bioactivity.

The XE-352 resin-containing cultures exhibited the highest phenol removal rates on a reactor liquid volume, support material mass, and estimated spherical surface area basis. Table 5.14 shows that the XE-352 resin concentration was approximately 3 times smaller than the activated carbon concentration.

The cultures containing activated carbon exhibited the highest phenol removal rates on a support material pore volume and surface area basis. These rates take into account the actual internal pore capacity and surface area of the support materials.

The bioactivity of the XE-352 resin-containing cultures had not yet reached their full potential at the time the assay was terminated (after 88 days incubation culture I). Figure 5.21 shows the phenol removal rate had not reached a plateau, indicating that the biofilm growth on the resin was still increasing. This is in contrast to what was observed in the F300 activated carbon cultures (Table 5.11), which showed that the bioactivity did not increase after the first consecutive supernatant exchange (day 75 of incubation). This suggests that the biomass growth on F300 activated carbon had reached its full potential. The XE-352 resin-containing cultures had 2.2 times the pore volume per bottle and 4.5 times the surface area per bottle than what was used in the activated carbon cultures (Table 5.14). The fact that the resin-containing cultures had more available pore volume and surface area for biomass growth may have accounted for the continuing biomass growth on the resin. The phenol removal rates per unit pore volume and surface area for the XE-352 resin-containing cultures (Table 5.15) may not be representative of the full phenol removal potential. That is to say, when biomass completely fills the pore capacity of the XE-352 resin, the phenol removal rates (per unit pore volume and surface area) will likely be higher than what was observed with the cultures containing activated carbon.

The bioactivity of the 76% supernatant cultures derived from the cultures containing activated carbon was substantially higher than XE-352 resin-containing cultures. The phenol removal rate of the activated carbon 76% supernatant cultures was 150 ± 39 mg/L•d, whereas, the resin 76% supernatant cultures removed phenol at 64 ± 15 mg/L•d. This is interpreted as a greater amount of biofilm loss occurring in the cultures containing activated carbon between supernatant exchanges. If the biomass growth had reached full capacity in the activated carbon cultures, as earlier suggested, and no pore volume or surface area capacity was available for further development, the biomass would continue to grow outwards in the bulk liquid. This biomass would be at greater risk to detachment from liquid hydraulic shearing forces and inter-particle abrasion. The biomass growth on the XE-352 resin may have not yet used up the available pore volume or surface area, and therefore, was not subjected to the same phenomenon.

5.5 Characterization of Support Materials For Biomass

5.5.1 Radiolabelled Cell Adsorption Studies

Pseudmonas aeruginosa are gram negative, aerobic bacteria (Nester et al., 1983) and their cell surface carries a net negative charge. Because this organism is commonly found in aerobic activated carbon columns treating drinking water (Burlingame et al., 1983) and it is much easier to grow than strict anaerobes, it was used to study the initial microbial adsorption to the three support materials. Suspensions of ^{14}C-labelled *P. aeruginosa* were contacted with different concentrations of the support materials. The equilibrium behavior of the *P. aeruginosa* cells was interpreted according to the Freundlich isotherm equation (Equation 5-3).

The adsorption behavior of *P. aeruginosa* cells to the different support materials is shown in Figure 5.25. Information used in deriving Figure 5.25 is summarized in Appendix XI. There was no organic carbon source present during the isotherm experiments (see section 3.5.1.2), therefore the initial number of *P. aeruginosa* cells was assumed to be constant over the 2 (or 4) h contact period.

The fitted Freundlich equations are:

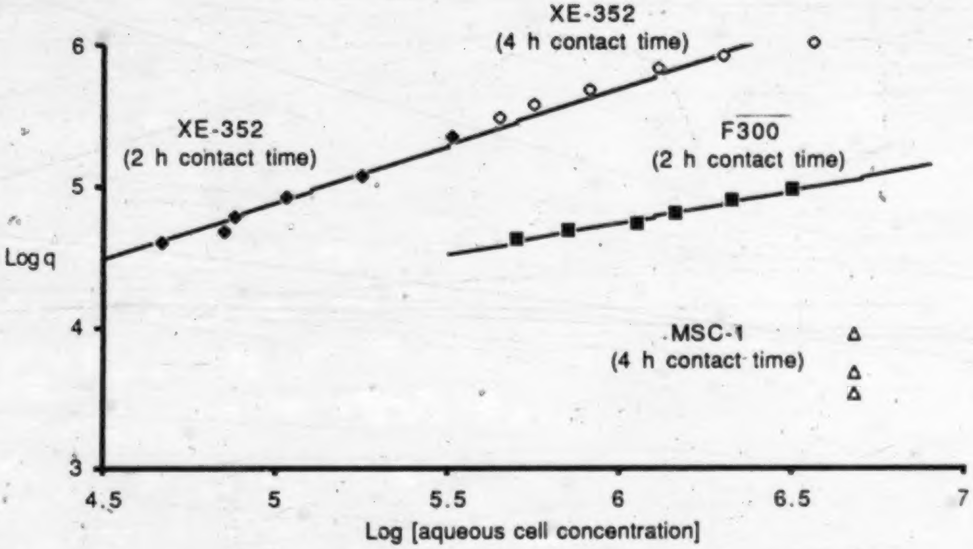


Figure 5.25 Adsorption Behavior of *Pseudomonas aeruginosa* Cells to Support Materials, Fitted to the Freundlich Equation

i) F300 activated carbon: $q = 80.1c_e^{0.47}$, (Eqn. 5-21)

95% confidence intervals: $14.7 \leq k \leq 145.6$,
$0.42 \leq \frac{1}{n} < 0.54$.

ii) XE-352 anion exchange resin: $q = 16.7c_e^{0.75}$. (Eqn. 5-22)

95% confidence intervals: $0.47 \leq k \leq 32.9$,
$0.68 \leq \frac{1}{n} < 0.83$.

Where: $q = \frac{\text{\# of adsorbed cells}}{\text{mg support material}}$, and

$$c_e = \frac{\text{\# of non-adsorbed cells}}{\text{mL solution}}.$$

The adsorption behavior of *P. aeruginosa* cells to MSC-1 resin indicated that the cells had little affinity for the cation exchange resin during the assay (4 h contact time). Table XI.3 (Appendix XI) shows that increasing the resin concentration did not reduce the concentration of cells in solution. The adsorption of cells did not increase with increasing resin concentration. This suggests that the adsorption behavior was independent of the resin concentation for the cell concentration tested. This is in general agreement with the results of the anaerobic phenol assay. The phenol removal capability of supernatant cultures was shown to be greater than cultures containing the resin (Figure 5.17b and 5.17c). The activity of the 12% supernatant cultures was superior to the resin cultures after the 1st supernatant exchange (incubation day 21). These results do not provide any evidence that bacteria were attaching to the MSC-1 resin surface.

The Freundlich equation for the XE-352 resin is based on adsorption data from 2 and 4 h contact times. The initial cell concentration in the 4 h assay was 10 times that of the 2 h assay. Similar adsorption behavior of the *P. aeruginosa* cells over the 2 and 4 h period indicate that adsorption was rapid and complete in ≤2 h.

The Freundlich k value is a measure of the adsorption capacity. A higher $\frac{1}{n}$ value indicates greater sensitivity in *P. aeruginosa* cell loadings. Figure 5.25 shows that XE-352 resin possesses a higher adsorption capacity for *P. aeruginosa* cells under the experimental conditions tested. XE-352 resin has 6.4 times the pore volume/gram, and 13 times the surface area/gram than activated carbon (see Table 5.14). These features may have contributed to the higher cell loadings observed on the XE-352 resin, compared to activated carbon, at the cell concentration tested. Considering typical suspended biomass concentration of biological wastewater systems (i.e. $\geq 10^6$ cells /mL), XE-352 is a more favorable support material for biomass attachment. Toerien and Hattingh (1969) reported that methanogenic and obligate anaerobic non-methanogenic bacteria may be present in anaerobic digestors in numbers of $\geq 10^6$ to 10^8 cells/mL.

5.5.2 Scanning Electron Microscopy

The support materials were examined and photographed by scanning electron microscopy (SEM) at the end of each bioassay. The objective was to observe the surface and attached-biomass characteristics. Each support material was also examined and photographed without bacteria. These samples of support materials were equilibrated in the growth medium mixture (see section 5.3.1.1) for 3 to 5 days prior to viewing.

MSC-1 Cation Exchange Resin

The surface of MSC-1 resin from the bioassay is shown in Figure 5.26. The resin was coated with a layer of debris (Figure 5.26a) that was presumably from the growth medium mixture. No evidence of bacterial growth was observed. The large clumps on the resin surface were viewed at higher magnification and found to be free of bacteria (Figure 5.26b). It can clearly be seen that the surface is smooth and contains no irregularities (i.e. pits, crevices, and pores) that would shelter bacterial growth. The pore volume characteristics of this resin (Figure 5.8) indicate no pore capacity is available to accommodate the anaerobic bacteria. The resin surface area (Table VII.3) is minimal compared to the other support materials (Table 5.14).

The only available surface area for this resin was the outer surface. Although no evidence of bacteria was observed on the surface, it does not imply bacteria were not able to establish themselves there. Chang and Rittmann (1986) reported that preparation of support materials for SEM is harsh for the attached-biomass. The loss of bacteria from surface extremities during preparation of the resin may have accounted for the absence of bacteria on the surface. However, the presence of substantial

(a) Bar = 100 µm

(b) Bar = 10 µm

Figure 5.26 MSC-1 Resin from Anaerobic Bioassay

amounts of debris on the surface suggest that if bacteria had become established on the surface, some at least would have been observed in the SEM photographs.

Results from SEM support the observations of the ^{14}C-labelled *P. aeruginosa* cell adsorption study and the anaerobic bioassay. The *P. aeruginosa* cells had very little adsorptive affinity for the surface. The anaerobic bioassay indicated that little or no bioactivity had become established on the surface after 21 days of incubation.

The lack of surface irregularities, pore volume (that would accommodate and shelter bacterial growth), and small surface area make MSC-1 resin a poor choice for use as a biological support. The resin did not exhibit favorable qualities for the adsorption of negatively charged aerobic *P. aeruginosa* cells to its surface. Also, factors that promote the removal of biomass from the surface (hydraulic shear and inter-particle abrasion) would play an important role in preventing adequate development of attached-biomass on the resin surface.

Selle Sardi et al. (1986) reported that strongly acidic functional groups on cation exchange resins was a preferred surface property for anaerobic biomass development due to the favorable characteristic of divalent cation binding and adsorption of volatile organic acids. The MSC-1 resin contained the same strongly acidic functionality (Figure 5.7b) as that used by Selle Sardi et al. (1986). In contrast, MSC-1 lacked qualities for the attachment of negatively-charged *P. aeruginosa* cells and the development of attached-anaerobic phenol-degrading biomass. One would expect the negatively-charged *P. aeruginosa* cells to have an affinity for the cation exchange sites on the MSC-1 resin. However, it may be that the unfavorable physical morphology of the resin (i.e. absence of surface irregularities) was a more important factor influencing the ability of biomass to attach to the surface.

F300 Activated Carbon

The surface of F300 activated carbon without biological growth is shown in Figure 5.27a. The obvious holes, crevices, and ridges are sites where attached bacteria would be protected from mechanisms that promote their removal from the surface. The surface contains many irregularities compared to the MSC-1 resin. Figure 5.27a shows the accumulation of debris from the growth medium mixture on all parts of the surface and in the pores. The debris did not aggregate in a layer on the activated carbon surface similar to what was observed on the MSC-1 resin surface (Figure 5.26a).

Figure 5.27b shows activated carbon with anaerobic microbial growth. It is interesting to note that massive biological growth is observed in many of the large pores, and little or no bacteria are evident on the outer surface of activated carbon. Also, the outer surface of the activated carbon at the pore entrances was relatively free of bacteria. Again, it must be mentioned that loss of bacteria during SEM preparation may have accounted for the absence of bacteria on the surface and at the pore entrances. However, the massive biological growth observed in the pores suggests that substantial biomass growth was able to establish itelf. This growth would account for the reduction in the apparent rate of phenol adsorption and adsorption capacity that was observed in Figure 5.14.

(a) Bar = 100 µm

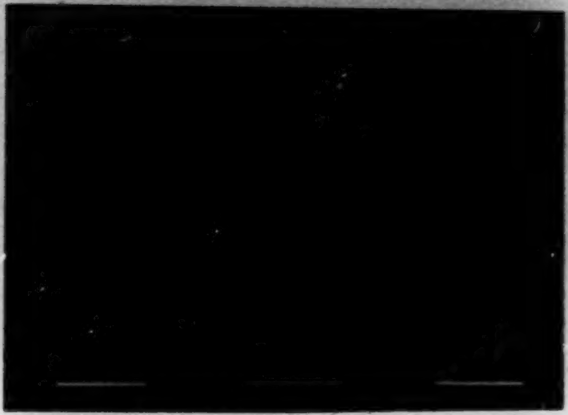
(b) Bar = 100 µm

Figure 5.27 F300 Activated Carbon (a) Free of Bacteria
(b) From Anaerobic Bioassay

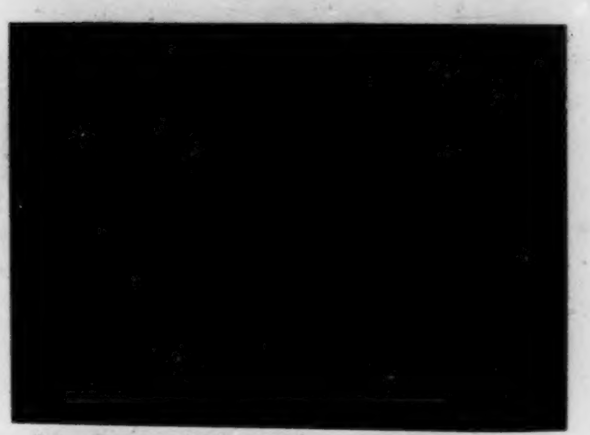
Figures 5.28 and 5.29 are more observations of biological growth on the F300 activated carbon. Samples from all three replicate serum bottle cultures in the anaerobic bioassay were viewed to ensure the phenomenon observed was consistant throughout. Figures 5.28b,c (and 5.29b,c) show views of biological growth in the pores enlarged from Figure 5.28a (and 5.29a). Figure 5.28b and c show a dominance of filamentous bacteria with a small cluster of short rod-shaped bacteria enmeshed in the center of the filamentous bacteria. Figure 5.29b and c show the presence of filamentous, rod-shaped, and cocci organisms all enmeshed in a mass of glycocalyx. The resulting consortium would be partially responsible for the conversion of phenol (the sole carbon source) to end products of methane and carbon dioxide gas (see section 2.1.2).

The pores of activated carbon appear to offer excellent sites for the attachment and colonization of biomass. Inter-particle abrasion and hydraulic shear would have little effect on biomass growth in the pores. The observation that bacteria appeared to be growing right out of the pores towards the bulk liquid (Figure 5.27b and 5.28a) may in part explain the high bioactivity exhibited by the 76% supernatant cultures (Table 5.11). If the biomass growth had reached full capacity in the activated carbon pores, and no pore volume or surface area capacity was available for further development, the biomass would continue to grow outwards in the bulk liquid. This biomass would be at greater risk to surface detachment from liquid hydraulic shearing forces and inter-particle abrasion.

An important observation in Figures 5.27, 5.28, and 5.29 is that a true biofilm did not cover the activated carbon particle surface,



(a) Bar = 100 µm

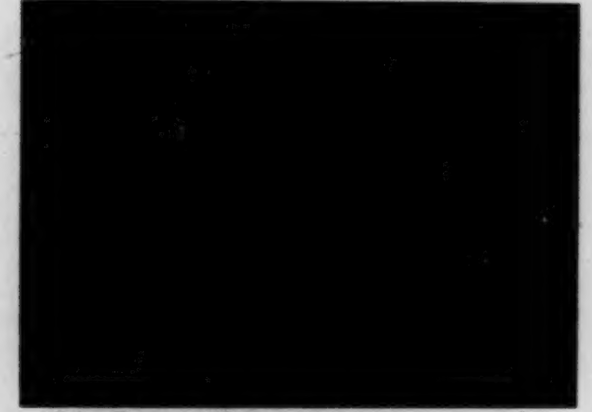


(b) Bar = 100 µm

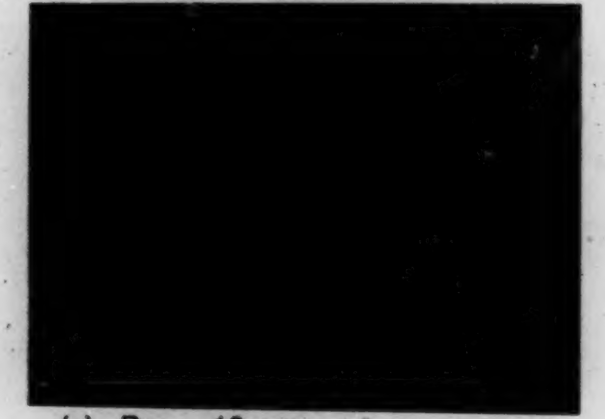
(c) Bar = 10 µm

Figure 5.28 F300 Activated Carbon from Anaerobic Bioassay

(a) Bar = 100 µm



(b) Bar = 10 µm



(c) Bar = 10 µm

Figure 5.29 F300 Activated Carbon from Anaerobic Bioassay

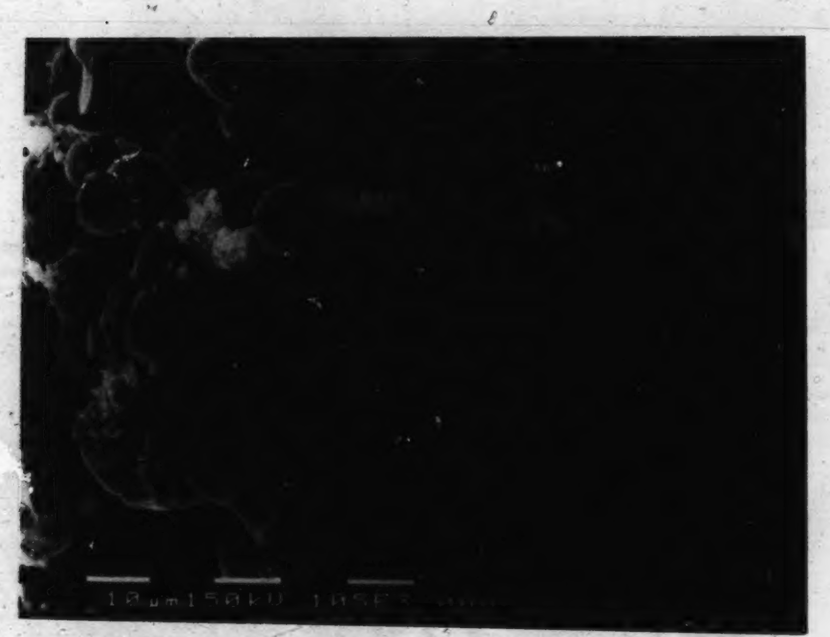
only biomass was evident in the pores. It is likely that the biomass growth period (≈100 days) was not sufficient to allow biofilm formation to establish itself and cover the activated carbon surface. It is important to point out that the previous and subsequent discussion on attached-biomass activity in the serum bottle bioassay with activated carbon is associated with bacteria in the pores of the activated carbon only. SEM clearly shows no evidence of biofilm on the activated carbon particle surface. The conditions examined and tested in the bioassay with activated carbon did not address biological activity associated with a biofilm.

The presence of surface irregularities, numerous pores (large enough to accommodate and shelter biomass growth), and surface area make F300 activated carbon an excellent choice as a biological support for the rapid development of attached-biomass. The F300 exhibited favorable qualities for the adsorption of negatively-charged aerobic *P. aeruginosa* cells to its surface. Also, mechanisms that promote the removal of biomass from the surface (hydraulic shear and inter-particle abrasion) would not have a major effect on biomass developing in the pores. However, once the biomass has utilized all the favorable pore and surface area capacity, eventual growth outwards in the bulk solution will expose the biomass to a greater risk of removal.

XE-352 Anion Exchange Resin

The surface of XE-352 anion exchange resin without biological growth is shown in Figure 5.30a. The spherical resin material is made from the fusion of smaller micro-spheres. The resulting

(a) Bar = 100 µm



(b) Bar = 10 µm

Figure 5.30 XE-352 Resin Free of Bacteria

structure exhibits high porosity. The resin internal pore volume and surface area available for bacterial colonization was substantially higher than for F300 activated carbon (see Table 5.14). Figure 5.30b is a magnified view of the resin showing the micro-spheres and immense pore structure. The voluminous pores provide numerous sites for attachment, and developing biomass would be sheltered from mechanisms that would promote their removal from the surface. The surface is highly irregular compared to the MSC-1 resin and appears smoother than the activated carbon surface. Figure 5.30b shows the accumulation of debris from the growth medium mixture on all parts of the surface.

Figure 5.31a shows the resin with anaerobic microbial growth. Massive biological growth is observed in all of the openings (compare with Figure 5.30b). Figure 5.31b shows the high concentration of glycocalyx material that was common on the surface. Filamentous, rod-shaped, and cocci organisms were all enmeshed in the glycocalyx.

Figure 5.32 shows more photographs of biomass on the resin surface. The depth to which the biomass penetrated in the porous structure would have been dictated by the internal pore dimension and availability of substrate (phenol). Diffusion of substrate into the biofilm would be responsible for providing the carbon source to biological growth deep in the pores. The simple comparison of phenol adsorption kinetics to activated carbon, in the absence and presence of biological growth, demonstrated the degree to which biological growth reduces the mass flux of phenol in the pores. The biomass growth in the pores simply impedes, or slows down, the free passage

(a) Bar = 100 µm

(b) Bar = 10 µm

Figure 5.31 XE-352 Resin from Anaerobic Bioassay

(a) Bar = 100 µm

(b) Bar = 10 µm

(c) Bar = 10 µm

Figure 5.32 XE-352 Resin from Anaerobic Bioassay

of phenol through the pores. The XE-352 resin does not have the advantage of phenol adsorption to provide a constant supply of carbon source throughout the pores, and therefore, may not have active biomass penetrated to the same depth as in the activated carbon pores.

The anaerobic bioassay with the resin indicated that attached-growth bioactivity was still increasing when the bioassay was terminated (Figure 5.21). The photograph in Figure 5.32a supports this observation. Figure 5.32a shows that the biomass growth had not yet completely filled up the pore capacity and surrounded the resin micro-spheres. This may partially explain the lower bioactivity associated with the 76% supernatant cultures from the resin assay compared with the activated carbon assay. The resin-attached biomass may not have been subjected to the same degree of removal mechanisms as the activated carbon-attached biomass because the resin-attached biomass did not extend as far out in the bulk solution and was still offered protection in the pores.

The abundance of huge pores (large enough to accomodate and shelter biomass growth from adverse removal mechanisms) make XE-352 resin an excellent choice as a biological support for the rapid development of attached-biomass. The resin exhibited favorable qualities for the adsorption of negatively-charged aerobic *P. aeruginosa* cells to its surface. Also, mechanisms that promote the removal of biomass from the surface (hydraulic shear and inter-particle abrasion) would not have a major effect on biomass developing in the large pores. The majority of biomass removed would not be associated with the resin surface, rather, it would be associated with glycocalyx and other biomass to which it is attached. The full extent of biomass loss was not completely addressed as the biofilm had not become fully developed on the resin when the bioassay was terminated.

5.6 Summary

The conditions examined and tested in the anaerobic bioassays with different biological supports addressed the behavior of attached-biomass in the early stages of development.

Dowex™ MSC-1 cation exchange resin (average size 300-850 µm) (Dow Chemical Co.) provided poor qualities as a biological support. Mercury porosimetry analysis of the resin revealed negligible pore capacity and surface area for the potential attachment and colonization of bacteria. The resin exhibited very poor adsorptive affinity for negatively-charged *P. aeruginosa* cells. A suspension of anaerobic phenol-degrading bacteria did not establish colonies on the resin surface in incubated culture bioassays. Biological activity of the culture supernatant was shown to be greater than biological activity associated with the resin. SEM of resin from the bioassay revealed no evidence of bacteria on the surface.

Ambergard™ XE-352 anion exchange resin (size 300-850 µm) (Rohm and Haas Co.) exhibited favorable qualities as a biological support. Mercury porosimetry analysis of the resin revealed measurable pore volume capacity and surface area for the potential attachment and colonization of bacteria. The resin exhibited excellent adsorptive affinity for negatively-charged *P. aeruginosa* cells indicating favorable surface chemistry. Isotherm experiments with the *P. aeruginosa* cells did not show any substantial increase in adsorption capacity when the contact time was increased from 2 to 4 h. This suggests that adsorption of the cells was rapid and complete in a short period of time (≤2 h). XE-352 resin was able to achieve a higher loading of *P. aeruginosa* cells on its surface compared to F300 activated carbon at cell concentrations more typical of anaerobic waste treatment systems (i.e. $\geq 10^6$ cells/mL).

Suspensions of anaerobic phenol-degrading bacteria established colonies on the resin surface in incubated culture bioassays. Resin-attached bioactivity was still increasing when the bioassay was terminated after 88 days incubation, suggesting that the biomass was still growing on the resin. SEM of resin from the bioassay revealed massive biological growth in the resin pores. The attached-biomass surface area exposed to the bulk liquid was substantially greater than what was observed with attached-biomass in the activated carbon pores, yet biomass loss from the resin was less than from activated carbon. The resin-attached biomass may still have been protected to a greater degree from mechansims that promote biomass removal.

The resin-attached biomass exhibited a substantial reduction in phenol removal capacity after being starved of phenol for a 24 to 48 h period. This short period of non-feeding resulted in a loss of bioactivity that was not temporary, suggesting a reduction in the quantity of active biomass.

Suspended bacteria removed from the resin culture were more susceptable than the resin-attached bacteria to substrate inhibition from a spike concentration of phenol. Negligible inhibition was observed with resin-attached bacteria that was spiked with a phenol concentration of 1,050 mg/L. However, the suspended bacteria became inhibited when fed a phenol dose of only 860 mg/L. The resin-attached and suspended bacteria had been previously fed 770 mg/L phenol prior to removing the supernatant and spiking with phenol. The resin-attached bacteria may have been protected in the surface pores from the higher spike phenol concentration (1,050 mg/L).

Filtrasorb® 300 activated carbon (size 420-850 µm) (Calgon Carbon Corp.) exhibited favorable qualities as a biological support for the rapid development of attached-biomass. Mercury porosimetry analysis of the activated carbon revealed measurable pore volume capacity and surface area for the potential attachment and colonization of bacteria. The activated carbon exhibited an adsorptive affinity for negatively-charged *P. aeruginosa* cells indicating favorable surface chemistry.

Suspensions of anaerobic phenol-degrading bacteria established colonies in the pores of activated carbon surface during incubated culture bioassays. Activated carbon surface-attached bioactivity did not increase after 75 days incubation, also supernatant removed from cultures containing activated carbon possessed substantially more biological activity than supernatant removed from cultures containing XE-352 resin. These observations suggest that increases in biomass growth on the activated carbon surface were offset by biomass removal mechanisms.

SEM of activated carbon from the bioassay revealed massive biological growth in the pores. This growth was likely responsible for the decrease in both the apparent rate and capacity of phenol adsorption which was documented. The adsorption capacity of F300 activated carbon provided a mechanism for removing phenol from solution that was absent in the resin bioassays. Bacteria in the activated carbon cultures were able to degrade phenol that was previously adsorbed to the activated carbon, demonstrating the bioregeneration capacity of the activated carbon-biomass system under dynamic substrate (phenol) loading conditions.

6.0 OVERALL SUMMARY AND CONCLUSONS

An effective approach in satisfying the treatment requirements of a high strength phenolic wastewater is to employ a combination of physical/chemical/biological processes. Biological treatment alone was impractical for the treatment of a coal liquefaction wastewater from the H-coal process because of inhibitory phenol concentrations (≥4,850 mg/L) and the presence of non-phenolic inhibitory compounds. However, organic solvent extraction accompanied by anaerobic biological treatment effectively removed high phenol concentrations in the H-coal wastewater. Adjusting the pH of H-coal wastewater and extracting with diisopropyl ether solvent successfully reduced the phenol concentration to a level amenable to anaerobic bacteria, and removed non-phenolic compounds previously shown to be inhibitory to the bacteria. The weakly acidic nature of phenol and substituted phenols allows for their selective removal by solvent extraction.

Diisopropyl ether was neither inhibitory to anaerobic bacteria, nor was it biodegraded to methane. The presence of water soluble diisopropyl ether did represent an excess chemical oxygen demand in the wastewater. Air stripping of anaerobically treated H-coal wastewater completely removed diisopropyl ether. Suspensions of anaerobic bacteria were able to actively degrade phenol in the solvent extracted wastewater under batch and semicontinuous loading conditions. However, the anaerobic bacteria exhibited instability during semicontinous feeding of the extracted wastewater. The addition of activated carbon to the stressed phenol-degrading cultures improved their ability to remove phenol from solution. Also, controlling the phenol feed demonstrated the unstable anaerobic cultures could be nursed back to efficient operation.

Further investigation into the role activated carbon performed during anaerobic phenol treatment demonstrated its importance as a biological support, in addition to providing adsorptive capacity for organic (including inhibitory) compounds. The similar study of other materials (ion exchange resins) which did not possess an adsorptive capacity for organic compounds supported these findings.

Difficulties were encountered in characterizing the biomass quantity associated with a biological support. This led to the adoption of criteria in which to assess the performance of the support materials. Physical evaluation methods, performance bioassays and radiolabelled cell adsorption studies (quatitative), and SEM (qualitative) were used to judge the value of the materials as biological supports.

Filtrasorb® 300 activated carbon provided favorable qualities as a biological support for the rapid development of attached-biomass. The irregular surface, internal porous structure and associated surface area of activated carbon provided superior colonization of anaerobic bacteria. However, considering the size of the bacteria, only a very small portion of the measurable surface area is available for colonization. Anaerobic phenol-degrading bacteria established well-developed growth in the pores of activated carbon. This growth was responsible for a reduction in both the apparent rate and extent of phenol adsorption to the activated carbon.

The adsorption capacity of activated carbon repeatedly removed spike concentrations of phenol from aqueous solution (via adsorption). Anaerobic bacteria were responsible for the continuous renewal of the adsorption capacity by degrading previously adsorbed phenol demonstrating the bioregeneration capacity of the activated carbon-biomass system under dynamic substrate (phenol) loading conditions.

Biomass growth associated with the activated carbon surface reached a maximum sooner than with the Ambergard™ XE-352 anion exchange resin surface under the conditions tested. Further increases in biomass growth on the activated carbon surface were offset by biomass removal mechanisms. This observation may be related to the smaller pore capacity and surface area available to bacteria on the activated carbon surface.

The Ambergard™ XE-352 anion exchange resin exhibited favorable qualities as a biological support for the rapid development of attached-biomass. This resin possesses substantially more pore capacity and surface area per dry gram than activated carbon to support biomass growth, and therefore, has the potential to achieve higher biomass loadings than activated carbon.

The resin-attached biomass exhibited a substantial reduction in phenol removal capacity after being starved of phenol for a 24 to 48 h period. This short period of non-feeding resulted in a loss of bioactivity that was not temporary, suggesting a reduction in the quantity of active biomass.

The resin-attached bacteria did not become inhibited from a spike concentration of phenol that was sufficient to inhibit suspended bacteria. The resin pores may have offered the attached-biomass protection from high phenol concentrations experienced in the bulk liquid.

The absence of pore capacity and surface area associated with Dowex™ MSC-1 cation exchange resin contributed to its poor performance as a biological support. The resin characteristics provided little or no potential for the attachment of anaerobic bacteria.

Excellent agreement was demonstrated among the physical evaluation methods, performance bioassays, radiolabelled cell adsorption studies, and SEM observations in judging the value of the materials as biological supports. A ranking, or preference, of desirable features that each support material possessed is summarized in Table 6.1. The pore capacity and associated surface area are the most important features that encourage the attachment and development of biomass on the surface. This successful evaluation allows one to set criteria to assess the preference of a support material that will provide optimum biomass development by characterizing the physical/chemical features that make an ideal biological support (i.e. pore capacity, surface area, substrate adsorptive capacity, and surface chemistry).

Table 6.1 Ranking of Desirable Features for the Different Biological Support Materials During the Early Stage of Biofilm Formation

Desirable Feature	F300 Activated Carbon	XE-352 Resin	MSC-1 Resin
Adsorptive Capacity[1]	Good	Poor	Poor
Surface Chemistry[2]	Good	Good	Neutral
Pore Capacity[3]	Good	Best	Poor
Surface Area[3]	Good	Best	Poor

[1] To provide the removal of excess levels of organic (substrate) and inhibitory compounds.

[2] To encourage the initial adsorption and attachment of bacteria to the surface.

[3] To shelter and protect attached-biomass, and to encourage attached-biomass persistence.

Activated carbon possessed all the desirable qualities of a biological support listed in Table 6.1 to enhance the treatment of H-coal wastewater. XE-352 resin did not possess any adsorptive capacity, however it contained more pore capacity and associated surface area per unit mass than activated carbon. These features alone may offer attached-biomass some degree of protection from organic (substrate) inhibition in the bulk solution and allow the treatment of an authentic wastewater like the H-coal wastewater during the early stages of biomass development on the resin surface. The ability of this resin to offer attached-biomass the same degree of protection from non-substrate inhibitory compounds is unknown. The MSC-1 resin would not provide any advantages as a biological support for the anaerobic treatment of the H-coal wastewater.

7.0 RECOMMENDATIONS FOR FUTURE WORK

Further study is needed to characterize the influence of pore capacity and associated surface area (available for the colonization of bacteria) of inert biological supports. These properties appeared to offer the best potential for attached-biomass development and persistence on the activated carbon and synthetic ion exchange resin surface in semicontinuous anaerobic phenol-degrading cultures. Specifically, the ability of these physical properties to offer attached-biomass protection from substrate and non-substrate inhibition experienced in the bulk solution should be investigated with non-adsorptive support materials.

It is important to characterize the pore capacity and associated surface area of a support material based on the dimensions of bacteria. Further research is recommended to investigate the depth to which bacteria can penetrate, attach, and colonize the pores of inert biological supports. This will establish the available pore capacity and associated surface area of support materials that are reported to have vast surface areas (e.g. activated carbon).

Anaerobic bioassays should be operated in the continuous mode to assess the pore capacity and associated surface area of support materials under steady-state conditions. This will provide the best information for the startup of full-scale biological treatment systems using inert biological supports.

Finally, a better understanding of the mechanisms that promote biomass removal from support materials is needed. Mechanisms such as fluid shear adversely affect biomass accumulation on support materials. This mechanism can have important consequences on accumulating biomass in fluidized-bed biological systems containing inert biological supports.

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Appendix I Theoretical Extraction Efficiency of Phenol From Pure Water With DIPE

The distribution ratio is:

$$D = \frac{K_c}{1 + K_a/[H^+]_w} \qquad \text{(Eqn. 4-19)}$$

At 25°C the acid dissociation constant for phenol (pK_a) is 10.0 (see Table 4.4). From Table 4.2 the experimental equilibrium distribution coefficient (K_c, concentration units) for phenol in a DIPE/water mixture at low pH and high dilution is 26.3. From Senetar and King (1986):

$$K_d = K_c\left(\frac{d_w}{d_s}\right), \text{ where} \qquad \text{(Eqn I-1)}$$

K_d = equilibrium distribution coefficient (weight fraction units),

d_w = density of water (0.9965 g/mL @ 25°C; Roberson and Crowe, 1975), and

d_s = density of DIPE (0.7182 g/mL @ 25 °C, Table 4.2).

Therefore:

$$K_d = 26.3\,\frac{\text{mg/L}}{\text{mg/L}} \cdot \frac{0.9965\ \text{g/mL}}{0.7182\ \text{g/mL}} = 36.5.$$

Equation (4-19) becomes:

$$D = \frac{36.5}{1 + 10^{-10}/[H^+]_w}\,. \qquad \text{(Eqn. I-2)}$$

Christian (1980) has shown that the percent of solute extracted (%) from water is related to the distribution ratio (D) by:

$$\%E = \frac{100D}{D + [V_w/V_s]} \qquad \text{(Eqn. I-3)}$$

where: V_w = aqueous phase volume; and
V_s = solvent phase volume.

A V_w/V_s ratio of 10 was used, therefore Equation (I-3) becomes:

$$\%E = \frac{100D}{D + 10}. \qquad \text{(Eqn. I-4)}$$

Equation (I-4) represents the percent phenol extracted for a single extraction. The percent phenol extracted for more than one extraction is determined from:

$$\%E_n = 100\% * [1 - (1 - \frac{\%E}{100})^n], \qquad \text{(Eqn. I-5)}$$

where n = number of extractions performed.

Equation (I-2) and (I-4) are used to determine the percent phenol extracted for a single extraction.

e.g. 1 For pH = 7 and $V_w/V_s = 10$:
Eqn. (I-2): D = 36.5,
Eqn. (I-4): %E = 78.5.

e.g. 2 For pH = 7 and $V_w/V_s = 2$:
Eqn. (I-2): D = 36.5,
Eqn. (I-3): %E = 94.8.

Equation (I-5) is used to determine %E for $n \geq 2$:

e.g. 3 For pH = 7, $V_w/V_s = 10$, and $n = 3$:

Eqn. (I-2): $D = 36.5$,

Eqn. (I-4): $\%E = 78.5$,

Eqn. (I-5): $\%E_{n=3} = 99.0$.

Appendix II Theoretical Chemical Oxygen (COD) Values for the Main Organics in Extracted H-coal Wastewater

1. The main organics in the pH 9 extracted wastewater are phenol and m/p-cresol. DIPE is soluble in water and may contribute to the organic loading. The theoretical COD of these organics are as follows:

i) phenol $C_6H_6O + 7O_2 \text{ ---> } 6CO_2 + 3H_2O$,

1 mg/L phenol = 2.38 mg/L COD.

ii) m/p-cresol $C_7H_8O + 8.5O_2 \text{ ---> } 7CO_2 + 4H_2O$,

1 mg/L m/p-cresol = 2.515 mg/L COD.

iii) DIPE $C_6H_{14}O + 9O_2 \text{ ---> } 6CO_2 + 7H_2O$,

1 mg/L DIPE = 2.819 mg/L COD.

From Table 4.3, the total COD of phenol and m/p-cresol in the pH 9 extracted wastewater are:

$$\text{phenol: } 1{,}050 \text{ mg/L} \cdot 2.38 \frac{\text{mg COD}}{\text{mg } C_6H_6O} = 2{,}499$$

$$\text{m/p-cresol: } 35 \text{ mg/L} \cdot 2.515 \frac{\text{mg COD}}{\text{mg } C_7H_8O} = 88$$

2,590 mg/L.

$$\frac{2{,}590}{[COD_{total} = 5{,}550]} \cdot 100 = 47\%.$$

2. The water solubility of DIPE is 0.9 %wt. @ 25°C (see Table 4.2). Therefore there is 0.009 g (or 9 mg) DIPE per 1 g of water and DIPE at maximum solubility. At 25°C:

density of water (d_w) = 0.9965 g/mL; and density of DIPE (d_s) = 0.7182 g/mL (see Appendix I).

Volume occupied by 9 mg DIPE is:

$$\frac{0\text{[illegible]}09\ \text{g}}{d_s} = \frac{0.009\ \text{g}}{0.7182\ \text{g/mL}} = 0.0125\ \text{mL}.$$

Volume occupied by 1 - 0.009 = 0.991 g water is:

$$\frac{0.991\ \text{g}}{d_w} = \frac{0.991\ \text{g}}{0.9965\ \text{g/mL}} = 0.9945\ \text{mL}.$$

Total volume is 0.0125 + 0.9945 = 1.007 mL.

Therefore, DIPE concentration is:

$$\frac{9\ \text{mg}}{1.007\ \text{mL}} * 10^3\ \frac{\text{mL}}{\text{L}} = 8{,}940\ \text{mg/L @ 25 °C}.$$

3. The COD of pH 9 extracted wastewater = 5,550 mg/L,
   COD of phenol and m/p-cresol = 2,590 mg/L,
   therefore, unaccounted COD = 2,960 mg/L.

Assuming that all of the unaccounted COD is DIPE:

$$\text{DIPE conc.} = [2{,}960\ \text{mg/L COD}] / [2.819\ \frac{\text{mg COD}}{\text{mg DIPE}}]$$
$$= 1{,}050\ \text{mg/L}.$$

Appendix III Concentration of Phenolics in Spent DIPE Fraction

The phenolic concentrations (mg/L) in the original un-extracted H-coal wastewater (pH 7) and pH 9 extracted wastewater are (Table 4.3):

	Phenol	m/p-Cresol	o-Cresol
Original waste	4,850	1,550	515
pH 9 extr. waste	1,050	35	0

The pH 9 wastewater was DIPE extracted three times at a wastewater to solvent ratio fo 10:1. Therefore 300 mL (in three separate 100 mL fractions) was used to extract every 1 L of the pH 9 wastewater. The estimated phenolic concentrations in the combined spent DIPE fraction are calculated from the extraction recovery information provided above.

For every liter of H-coal extracted at pH 9 the concentration of:

$$\text{Phenol} = \frac{4{,}850 - 1{,}050 \text{ mg}}{300 \text{ mL}} \cdot 10^3 \frac{\text{mL}}{\text{L}} = 12{,}670 \text{ mg/L},$$

$$\text{m/p-Cresol} = \frac{1{,}550 - 35 \text{ mg}}{300 \text{ mL}} \cdot 10^3 \frac{\text{mL}}{\text{L}} = 5{,}050 \text{ mg/L},$$

$$\text{o-Cresol} = \frac{515 - 0 \text{ mg}}{300 \text{ mL}} \cdot 10^3 \frac{\text{mL}}{\text{L}} = 1{,}720 \text{ mg/L}$$

in the extraction solvent.

Appendix IV Batch Fermentation of Solvent Extracted Wastewater

Table IV.1 Assay I - Batch Fermentation of Phenolics (750 mg/L Total Phenolics)

Phenolic Concentration (mg/L)

Incubation Day:		0	7	14	21	28	35	42
Culture	Compound							
pH 7.0	Phenol	550	425	278	92	0	0	0
Extr.	m/p-Cresol	160	150	103	109	64	61	32
H-coal	o-Cresol	50	40	33	29	30	32	29
pH 8.5	Phenol	545	400	200	10	0	0	0
Extr.	m/p-Cresol	152	146	110	102	49	44	18
H-coal	o-Cresol	47	42	32	26	30	27	28
pH 10	Phenol	617	460	256	0	0	0	0
Extr.	m/p-Cresol	124	119	75	57	43	35	0
H-coal	o-Cresol	28	22	15	15	15	15	15
pH 11.5	Phenol	562	450	386	113	0	0	0
Extr.	m/p-Cresol	151	123	110	90	69	59	45
H-coal	o-Cresol	38	32	30	23	24	24	25
Synthetic	Phenol	530	357	127	0	0	0	0
	m/p-Cresol	157	118	88	70	62	20	0
	o-Cresol	50	38	30	32	33	30	32

Table IV.2 Assay I - Batch Fermentation of Phenolics (500 mg/L Total Phenolics)

		Concentration (mg/L)				
Incubation Day:		0	7	14	21	28
Culture	Compound					
pH 7	Phenol	370	181	0	0	0
Extr.	m/p-Cresol	106	84	42	0	0
H-coal	o-Cresol	33	25	27	27	26
pH 8.5	Phenol	377	144	0	0	0
Extr.	m/p-Cresol	105	64	0	0	0
H-coal	o-Cresol	32	27	26	25	25
pH 10	Phenol	425	147	0	0	0
Extr.	m/p-Cresol	84	49	0	0	0
H-coal	o-Cresol	19	12	15	13	14
pH 11.5	Phenol	375	199	0	0	0
Extr.	m/p-Cresol	100	82	0	0	0
H-coal	o-Cresol	25	22	21	18	19
Synthetic	Phenol	360	40	0	0	0
	m/p-Cresol	105	70	0	0	0
	o-Cresol	33	25	26	22	22

Table IV.3 Assay II - Batch Fermentation of Phenolics (750 mg/L Total Phenolics)

		Concentration (mg/L)					
Incubation Day:		0	7	14	21	28	42
Culture	**Compound**						
pH 8.5	Phenol	523	413	215	0	0	0
Extr.	m/p-Cresol	146	119	123	72	58	25
H-coal	o-Cresol	50	43	45	39	34	36
pH 9	Phenol	553	410	156	0	0	0
Extr.	m/p-Cresol	129	107	92	61	56	0
H-coal	o-Cresol	43	38	34	35	31	31
pH 9.5	Phenol	608	407	252	0	0	0
Extr.	m/p-Cresol	116	76	75	55	45	0
H-coal	o-Cresol	33	23	23	25	24	27
pH 10	Phenol	541	444	103	0	0	0
Extr.	m/p-Cresol	106	86	69	59	53	0
H-coal	o-Cresol	33	22	18	21	19	21
Synthetic	Phenol	476	300	33	0	0	0
	m/p-Cresol	136	106	85	62	0	0
	o-Cresol	46	39	38	37	39	39

Table IV.4 Assay II - Percent Methane Accumulation in Culture Headspace[1]

	Methane Concentration (%)			
Incubation Day:	3	10	18	24
Culture:				
Extr. pH 8.5	12.8	23.2	34.1	38.5
Extr. pH 9	13.4	23.2	35.1	37.7
Extr. pH 9.5	12.4	21.6	32.2	37.8
Extr. pH 10	13.1	22.0	37.2	37.8
Blank	11.5	19.2	21.5	25.7
Synthetic	10.7	25.5	35.4	37.0

[1] Approximate total phenolic concentration = 750 mg/L @ day 0.

Appendix V Semicontinuous Fermentation of Solvent Extracted Wastewater

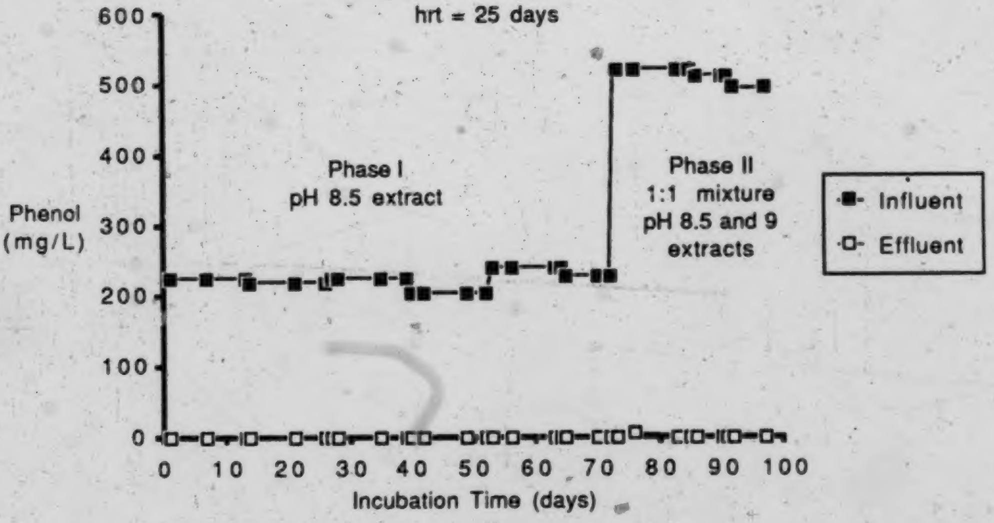


Figure V.1 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater - Phase I and II

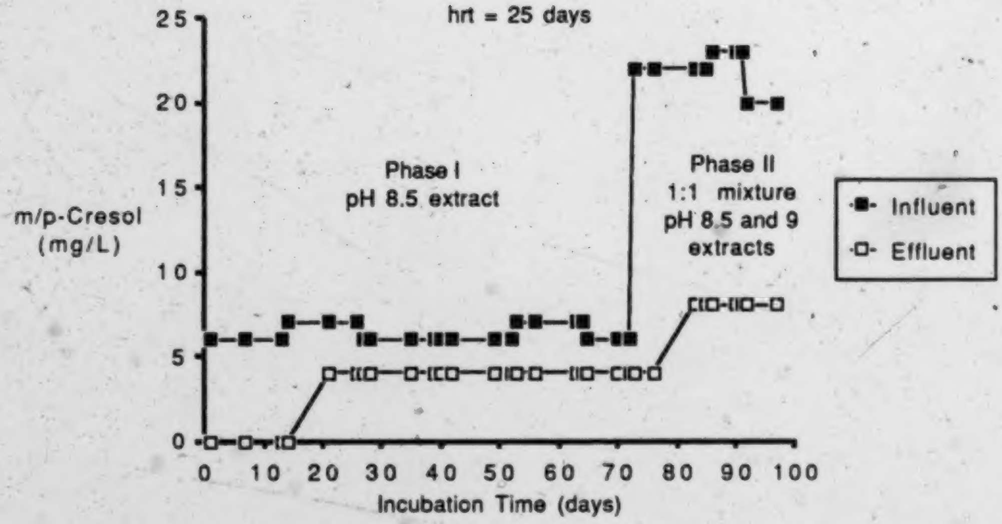


Figure V.2 Semicontinuous Fermentation of m/p-Cresol in Extracted H-coal Wastewater - Phase I and II

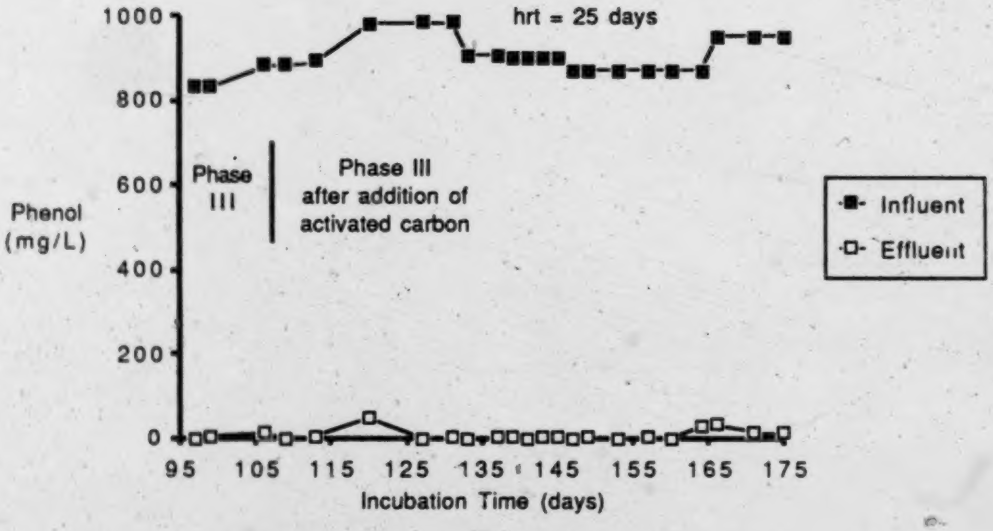


Figure V.3 Semicontinuous Fermentation of Phenol in Extracted H-coal Wastewater - Phase III

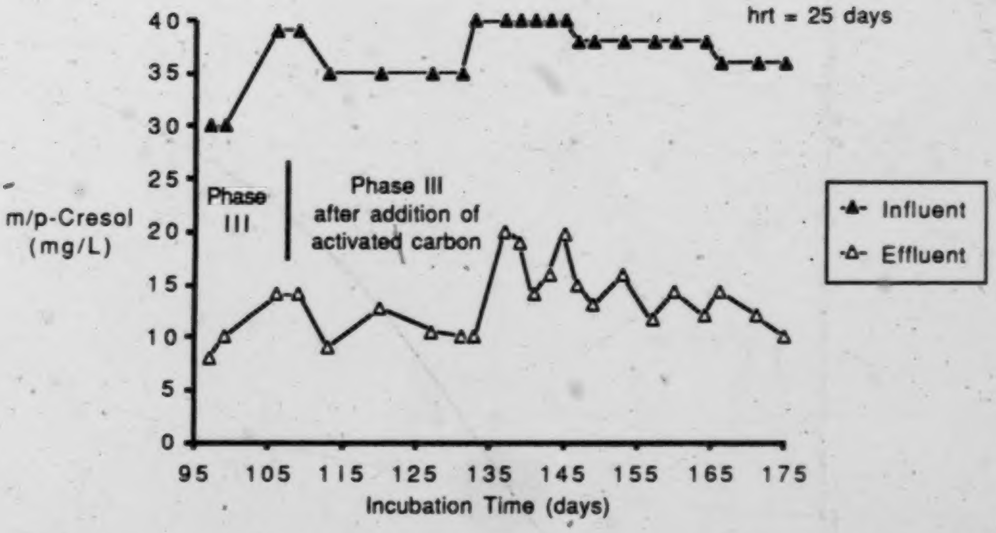


Figure V.4 Semicontinuous Fermentation of m/p-Cresol in Extracted H-coal Wastewater - Phase III

Appendix VI **Data Analysis of Semicontinuous Fermentation Studies**

1. Phase II m/p-Cresol Effluent Behavior

Given a step increase of feed to a non-reactive continuously stirred tank reactor (CSTR), the effluent behavior is given by:

$$C_t = C_o[1 - e^{-t/t_d}], \text{ where: } \qquad \text{(Eqn. VI-1)}$$

C_t = effluent concentration at time t after step increase (mg/L),

C_o = influent concentration (mg/L),

t = time after step increase (d), and

t_d = hydraulic residence time (d).

On day 73 the average feed concentration of m/p-cresol increased from 6 to 22 mg/L (a step increase of 16 mg/L), therefore for C_o = 16 mg/L and for t_d = 16.7 mg/L:

$$C_t = 16[1 - e^{-t/16.7}] + 4 \text{ (mg/L)}, \qquad \text{(Eqn. VI-2)}$$

where 4 mg/L represents the assumed steady-state effluent concentration prior to the step increase.

Comparison of the observed m/p-cresol concentration in the effluent from cultures maintained at a HRT of 16.7 d to the predicted washout concentration in the absence of any removal mechanisms is shown in Figure VI.1. If all mechanisms for m/p-cresol removal have stopped (simultaneously with the step increase), the effluent concentration should follow the predicted washout concentrations (based on equation VI-2).

The effluent m/p-cresol behavior, immediately following the step increase of influent (day 72-83), is similar to the washout curve. This suggests that m/p-cresol removal may have stopped

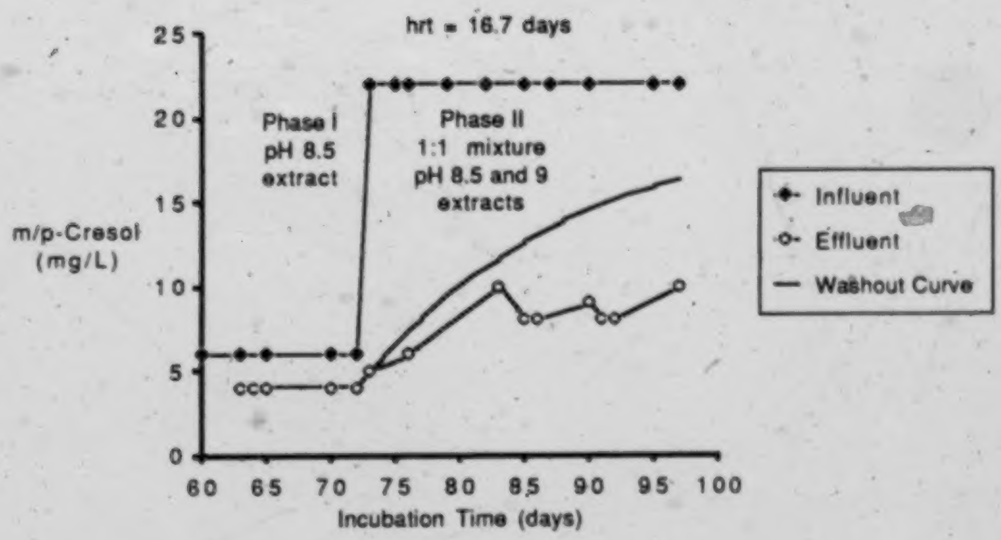


Figure VI.1 m/p-Cresol Effluent Concentration Compared to Washout Concentration - Phase I and II during this time. However, the obvious difference in the effluent and washout m/p-cresol concentrations after day 83 indicate that m/p-cresol removal was able to recover.

2. Methane Generation Efficiency Based on COD Equivalence and COD Feed Rates

a. COD Equivalence of methane (CH_4) is derived from:

$$CH_4 + 2O_2 \Longrightarrow CO_2 + H_2O. \qquad \text{(Eqn. VI-3)}$$

From Equation (VI-3) 1 mg O_2 (i.e. COD) = 0.25 mg CH_4.

$$V = \frac{nRT}{P}\text{, where:} \qquad \text{(Eqn. VI-4)}$$

V = volume (L),

n = moles of gas,

R = $0.082054 \dfrac{L\cdot atm}{mole\cdot {}^{o}K}$,

T = absolute temperature (°K), and

P = pressure (atm).

Therefore for:

1 mg CH_4 (as COD) = 0.25 mg CH_4 (as CH_4) = $1.56(10^{-5})$ moles CH_4,

T = 37°C (310.15°K), and

P = 1 atm,

from Equation (VI-4):

$$V = [1.56(10^{-5})\text{ moles} \cdot 0.082054 \frac{L\cdot atm}{mole\cdot {}^{o}K} \cdot 310.15^{o}K] / [1\text{ atm}]$$

$$= 3.98(10^{-4})\text{ L} = 0.398\text{ mL (0.4 mL)}.$$

Or, 0.4 mL of CH_4 at 37°C and 1 atm is equivalent to 1 mg COD:

1 mL of CH_4 = 2.5 mg COD. (Eqn. VI-5).

b. COD of Extracted H-coal Wastewater Feed:

Feed solutions were made with extracted H-coal wastewater (90%) and a synthetic nutrient medium (10%). Therefore from Table 4.3:

pH 8.5 extracted wastewater = 2,720 mg COD/L;

1:1 mixture pH 8.5/9 extracted wastewater = 4,135 mg COD/L (calculated); and

pH 9 extracted wastewater = 5,550 mg COD/L.

Accounting for 10% dilution from nutrient medium addition:

pH 8.5 extr. feed = 2,450 mg COD/L = 2.45 mg COD/mL;

pH 8.5/9 extr. feed = 3,720 mg COD/L = 3.72 mg COD/mL; and

pH 9 extr. feed = 5,000 mg COD/L = 5.00 mg COD/mL.

The COD feed procedures from Table 4.7 are:

Phase I:

25 day HRT: 2 mL/d • 2.45 mg COD/mL = 4.9 mg COD fed/d;

16.7 day HRT: 3 mL/d • 2.45 mg COD/mL = 7.4 mg COD fed/d.

Phase II:

25 day HRT: 2 mL/d • 3.72 mg COD/mL = 7.4 mg COD fed/d;

16.7 day HRT: 3 mL/d • 3.72 mg COD/mL = 11.2 mg COD fed/d.

Phase III (after activated carbon addition the culture liquid volumes were cut in half, and fed one half the feed volume to maintain the same HRT):

25 day HRT: 1 mL/d • 5.00 mg COD/mL = 5.0 mg COD fed/d;

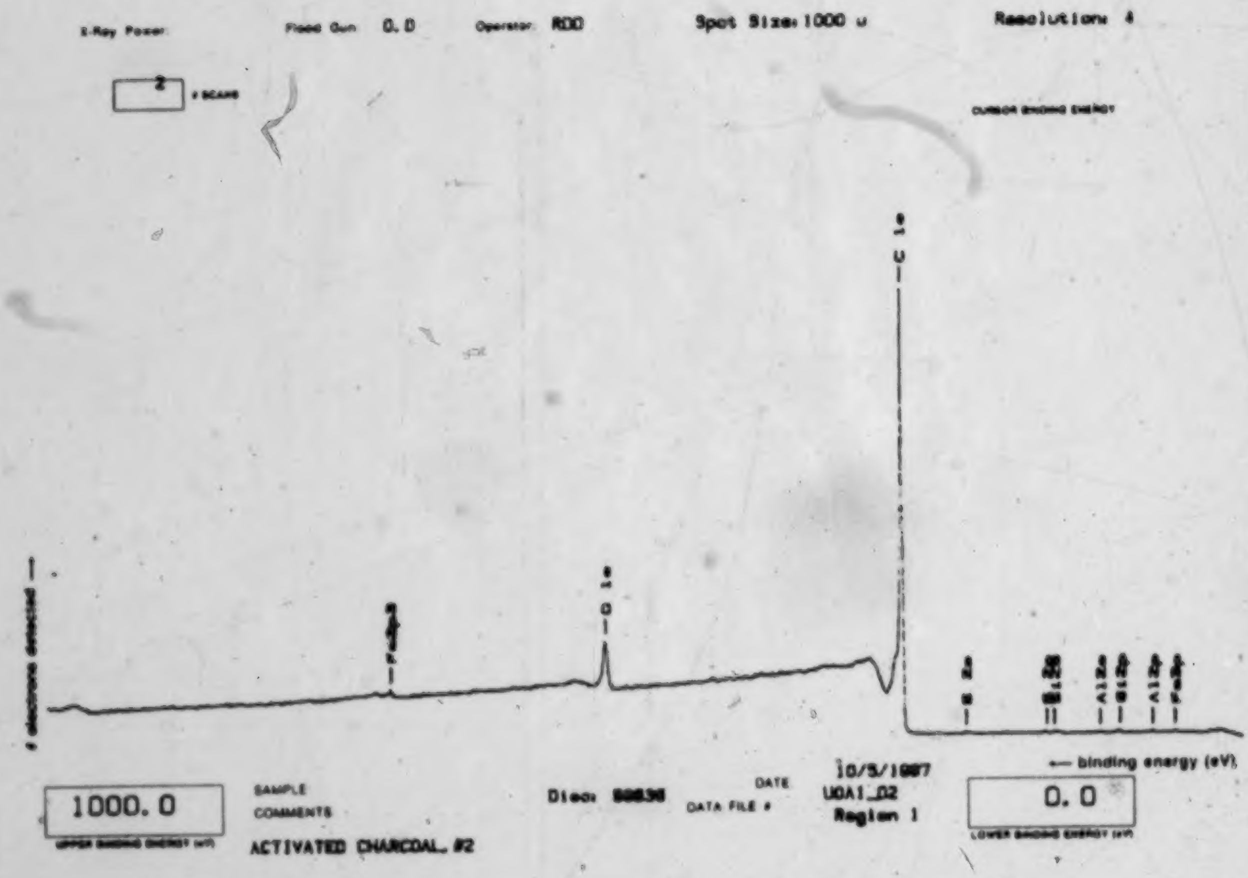
16.7 day HRT: 1.5 mL/d • 5.0 mg COD/mL = 7.5 mg COD fed/d.

Phase V:

12.5 day HRT: 2 mL/d * 5.00 mg COD/mL = 10 mg COD fed/d.

Because the reactor liquid volumes (and feed volumes) were reduced by one-half during Phase III, Table 4.11 provides a comparison of COD loading rates for each phase steady state operating periods.

Appendix VII Surface ,Characterization of Support Materials



X-Ray Power: Flood Gun: 0.0 Operator: RDD Spot Size: 1000 u Resolution: 4

2 # SCANS

1000.0

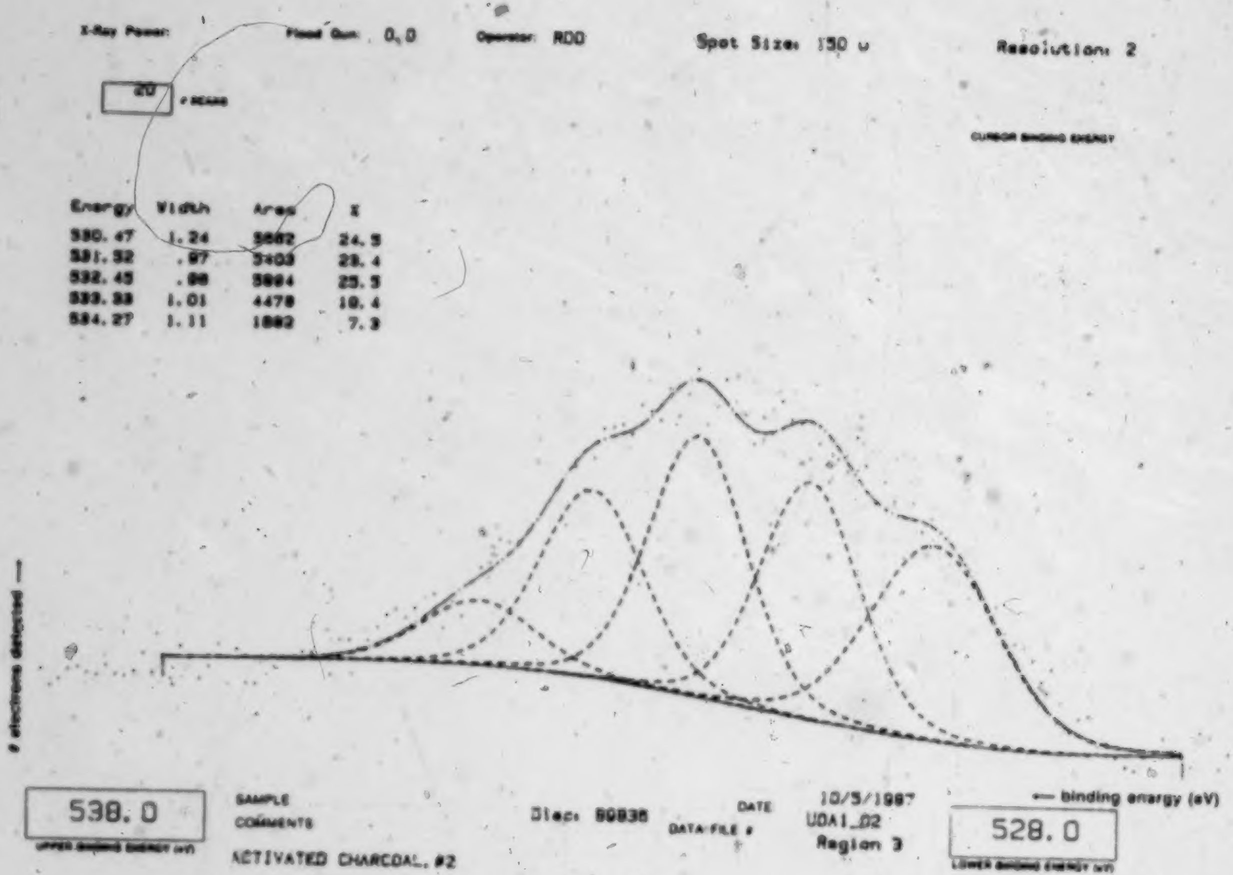
SAMPLE
COMMENTS
ACTIVATED CHARCOAL #2

Dieo: 88836

DATE 10/3/1987
DATA FILE # UDA1_02
Region 1

0.0

Figure VII.1 Widescan X-ray Photoelectron Spectra of F300 Activated Carbon (Vertical Scale: 5625 counts/cm)



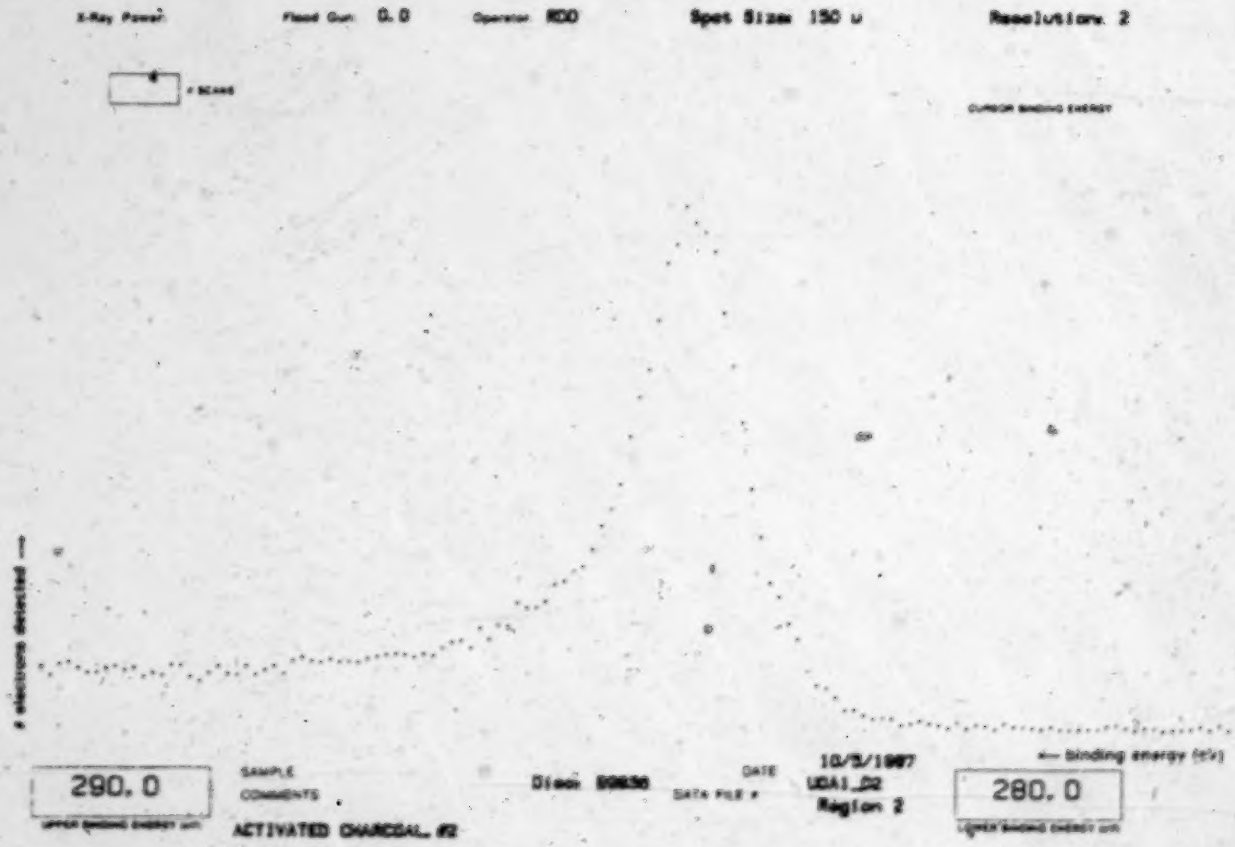
X-Ray Power: 20 # SCANS Flood Gun: 0.0 Operator: RDD Spot Size: 150 u Resolution: 2

Energy	Width	Area	%
530.47	1.24	5862	24.5
531.52	.97	5403	23.4
532.45	.88	5884	25.3
533.38	1.01	4478	18.4
534.27	1.11	1882	7.3

SAMPLE COMMENTS: ACTIVATED CHARCOAL, #2

Disc: 80838 DATE: 10/5/1987 DATA FILE #: UDA1_02 Region 3

Figure VII.2 High Resolution Narrow Scan X-ray Photoelectron Spectra on Oxygen (1s) Peak of F300 Activated Carbon (Vertical Scale: 112.5 counts/cm)



Figure, VII.3 High Resolution Narrow Scan X-ray Photoelectron Spectra on Carbon (1s) Peak of F300 Activated Carbon (Vertical Scale: 225 counts/cm)

Table VII.1 Pore and Surface Area Properties of F300 Activated Carbon

Mean Pore Diameter D (μm)	Cumulative Pore Volume V (cm^3/g)	$\Delta V/\Delta D$ ($cm^3/g \cdot \mu m$)	Cumulative Area	
			(m^2/g)	(%)
103.92	0.054	0	0.0022	0.003
72.74	0.091	0.001	0.0043	0.006
56.12	0.115	0.002	0.0061	0.008
45.72	0.131	0.002	0.0075	0.010
38.60	0.146	0.002	0.0091	0.012
33.40	0.154	0.002	0.0101	0.013
29.44	0.162	0.002	0.0112	0.015
26.33	0.167	0.002	0.0120	0.016
23.81	0.173	0.002	0.0130	0.017
21.73	0.176	0.001	0.0136	0.018
19.98	0.180	0.002	0.0143	0.019
18.50	0.183	0.002	0.0151	0.020
17.22	0.188	0.004	0.0162	0.021
16.11	0.190	0.002	0.0167	0.022
15.13	0.192	0.002	0.0173	0.023
7.04	0.197	0.001	0.0204	0.027
3.39	0.210	0.004	0.0385	0.050
1.95	0.224	0.010	0.0679	0.088
1.38	0.242	0.032	0.122	0.159
0.994	0.270	0.072	0.243	0.316
0.704	0.295	0.086	0.389	0.507
0.547	0.316	0.127	0.543	0.706
0.448	0.330	0.152	0.677	0.882
0.329	0.371	0.345	1.21	1.58
0.212	0.397	0.222	1.73	2.25
0.150	0.424	0.426	2.49	3.24
0.103	0.455	0.660	3.74	4.87
0.072	0.475	0.645	4.91	6.39
0.052	0.509	1.70	7.68	10.0
0.036	0.533	1.50	10.4	13.5
0.027	0.567	3.78	15.7	20.4
0.020	0.595	4.00	21.4	27.9
0.015	0.647	10.4	35.9	46.7
0.011	0.680	8.25	47.9	62.4
0.009	0.716	18.0	64.2	83.6
0.008	0.716	24	76.8	100

Table VII.2 Pore and Surface Area Properties of XE-352 Resin

Mean Pore Diameter	Cumulative Pore Volume	$\Delta V/\Delta D$	Cumulative Area	
D (μm)	V (cm^3/g)	(cm^3/g·μm)	(m^2/g)	(%)
93.53	0.038	0	0.0017	0.002
72.74	0.119	0.004	0.0063	0.009
56.12	0.189	0.004	0.0115	0.017
45.72	0.239	0.005	0.0159	0.024
38.60	0.282	0.006	0.0205	0.030
33.02	0.328	0.008	0.0261	0.039
28.76	0.364	0.009	0.0312	0.046
26.03	0.402	0.014	0.0372	0.055
23.81	0.451	0.022	0.0456	0.068
21.73	0.523	0.034	0.0591	0.088
19.98	0.612	0.052	0.0773	0.114
18.50	0.735	0.082	0.104	0.155
17.22	0.905	0.131	0.145	0.214
16.11	1.076	0.155	0.188	0.278
15.13	1.220	0.144	0.227	0.335
7.18	1.396	0.035	0.342	0.507
3.42	1.439	0.012	0.400	0.592
1.94	1.455	0.010	0.433	0.641
1.38	1.458	0.007	0.445	0.658
1.01	1.462	0.011	0.461	0.682
0.707	1.466	0.013	0.483	0.715
0.546	1.466	0	0.483	0.715
0.446	1.466	0	0.483	0.715
0.327	1.470	0.034	0.533	0.789
0.212	1.470	0	0.533	0.789
0.150	1.474	0.065	0.639	0.945
0.103	1.481	0.170	0.950	1.41
0.072	1.485	0.129	1.17	1.73
0.052	1.500	0.750	2.41	3.56
0.036	1.511	0.688	3.71	5.48
0.027	1.527	1.67	6.06	8.97
0.020	1.555	4.00	11.9	17.6
0.015	1.597	8.40	23.4	34.7
0.011	1.655	14.8	45.0	66.6
0.009	1.674	9.50	53.5	79.2
0.008	1.701	27.0	67.6	100

Table VII.3 Pore and Surface Area Properties of MSC-1 Resin

Mean Pore Diameter D (μm)	Cumulative Pore Volume V (cm^3/g)	$\Delta V/\Delta D$ ($cm^3/g \cdot \mu m$)	Cumulative Area (m^2/g)	(%)
72.74	0.002	0	0	0
56.12	0.004	0	0	0
45.72	0.005	0.002	0	0
38.60	0.006	0	0	0
33.40	0.006	0	0.0005	0.001
29.44	0.007	0	0.0006	0.001
26.33	0.007	0	0.0006	0.001
23.08	0.007	0	0.0006	0.001
21.73	0.007	0	0.0006	0.001
20.13	0.007	0	0.0006	0.001
18.65	0.007	0	0.0006	0.001
17.22	0.007	0	0.0006	0.001
16.11	0.007	0	0.0006	0.001
15.13	0.007	0	0.0006	0.001
7.00	0.007	0	0.0006	0.001
3.38	0.007	0	0.0006	0.001
1.93	0.007	0	0.0006	0.001
1.37	0.007	0	0.0006	0.001
0.998	0.007	0	0.0006	0.001
0.709	0.007	0	0.0006	0.001
0.550	0.007	0	0.0006	0.001
0.447	0.007	0.010	0.0092	0.016
0.324	0.008	0.016	0.0366	0.064
0.211	0.009	0.018	0.0772	0.134
0.150	0.012	0.049	0.163	0.283
0.103	0.017	0.106	0.373	0.649
0.072	0.022	0.129	0.608	1.06
0.052	0.035	0.700	1.74	3.03
0.036	0.211	10.94	21.8	37.9
0.027	0.286	8.33	33.4	58.1
0.020	0.311	3.57	38.6	67.0
0.015	0.335	4.80	45.2	78.7
0.011	0.347	3.00	49.8	86.5
0.009	0.355	4.00	53.5	92.9
0.008	0.363	8.00	57.5	100

Appendix VIII Spectrophotometry of Carbohydrate and Protein Standards

1. Carbohydrates

A 15 µg/mL carbohydrate (glucose) standard was used to develop an absorbance versus wavelength (nm) relationship. Figure VIII.1 demonstrates the absorbance characteristics of the standard near the chosen wavelength (485 nm). Dubois et al. (1956) recommended a wavelength of 485 nm for the measurement of sugars and related substances by the phenol-acid digestion method. Pentoses and methlypentoses have absorption maximums at 480 nm, while hexoses and their methylated derivatives have an aborption maximum at 485 to 490 nm. The carbohydrate content of anaerobic bacteria will contain a variety of carbohydrates, each with a different absorption maximum. The 485 nm wavelength was chosen as a measurement of total carbohydrates in samples containing mixtures of sugars and their methyl derivatives.

Figure VIII.1 shows that the absorption maximum for the glucose standard occurs at 492 nm, however the absorption characteristics for different glucose concentrations were always observed to be linear at 485 nm. Figure VIII.2 shows a typical calibration curve of glucose standards at 485 nm. A new glucose calibration curve was derived every time anaerobic sludge was analyzed for carbohydrates. This practice eliminates variations in instrument response and analytical technique over the course of the carbohydrate measurements.

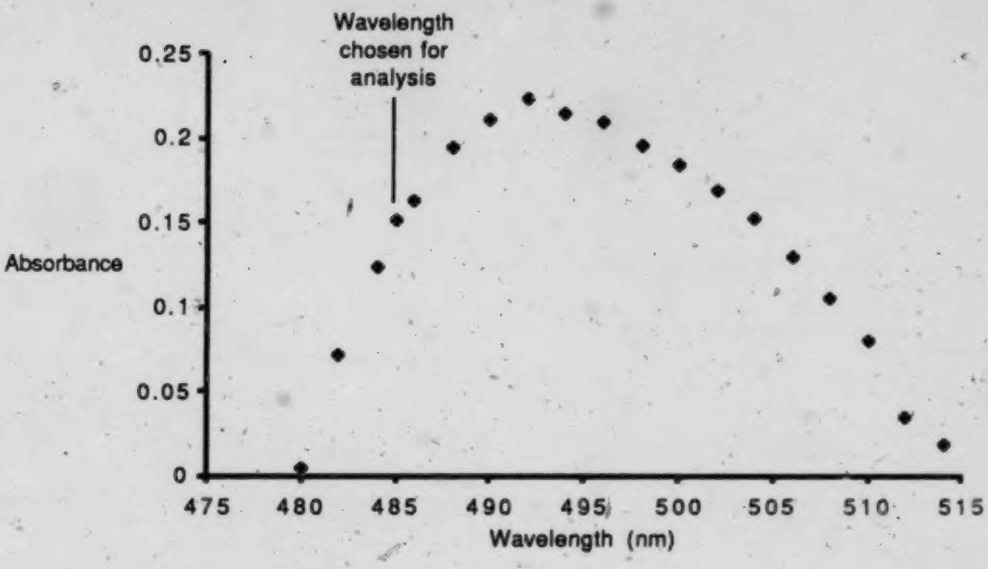


Figure VIII.1 Absorbance Spectrum of Glucose Standard

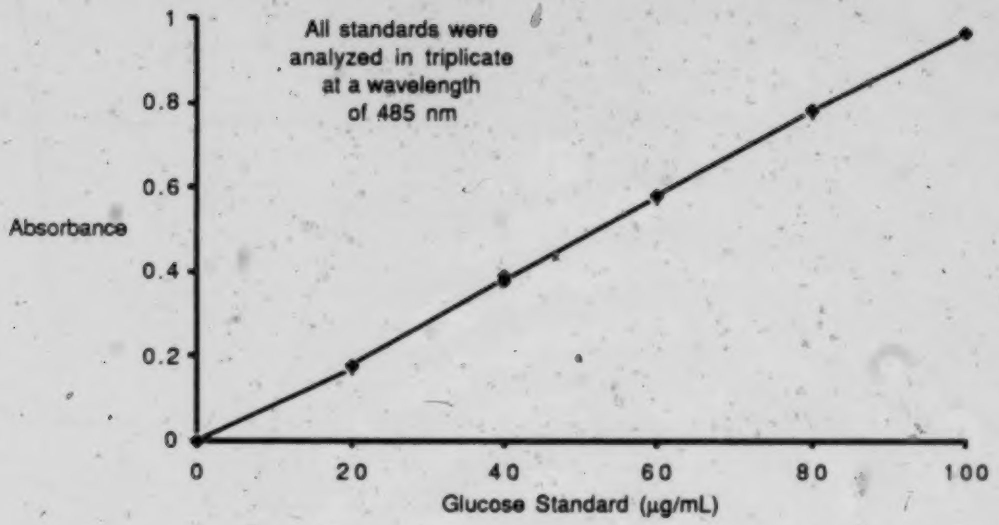


Figure VIII.2 Absorbance Calibration Curve for Glucose Standards

2. Proteins

Bovine plasma gamma gobulin (BPGG) was the recommended protein standard used for the quantitation of total proteins in anaerobic bacteria. A 23 µg/mL protein standard was used to develop an absorbance versus wavelength (nm) relationship. Figure VIII.3 demonstrates the absorbance characteristics of the BPGG standard. Bradford (1976) indicated that binding of Cromassie Brilliant Blue G-250 dye to proteins causes a shift in the absorption maximum of the dye from 465 to 595 nm. The absorption characteristics of the BPGG standard-dye complex in the region of 595 nm shows that an absorption minimum exists. However, the absorption characteristics for different BPGG standards were always observed to be linear at 595 nm. Figure VIII.4 shows a typical calibration curve of BPGG standards at 595 nm. A new calibration curve was derived every time anaerobic sludge was analyzed for proteins.

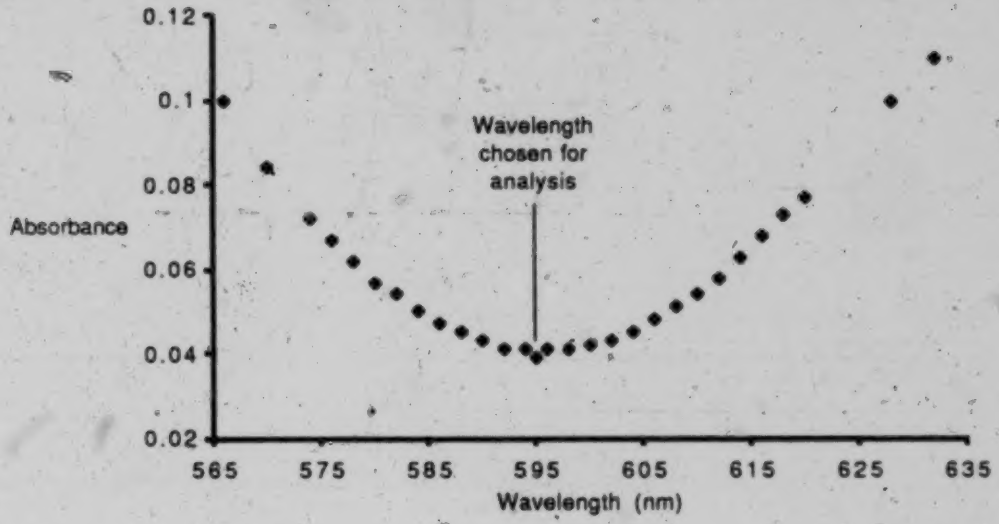


Figure VIII.3 Absorbance Spectrum of Protein Standard

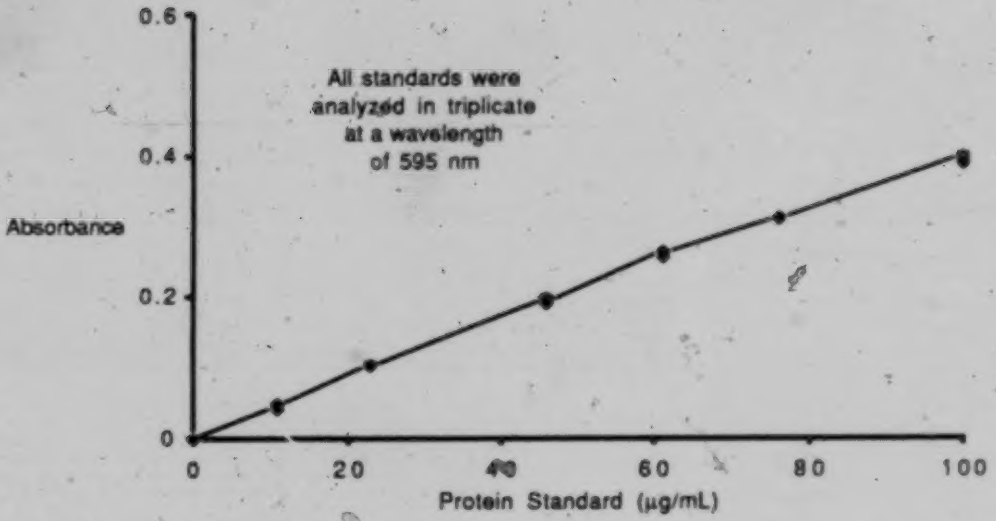


Figure VIII.4 Absorbance Calibration Curve for Protein Standards

Appendix IX Phenol Biodegradation in Cultures Containing F300 Activated Carbon

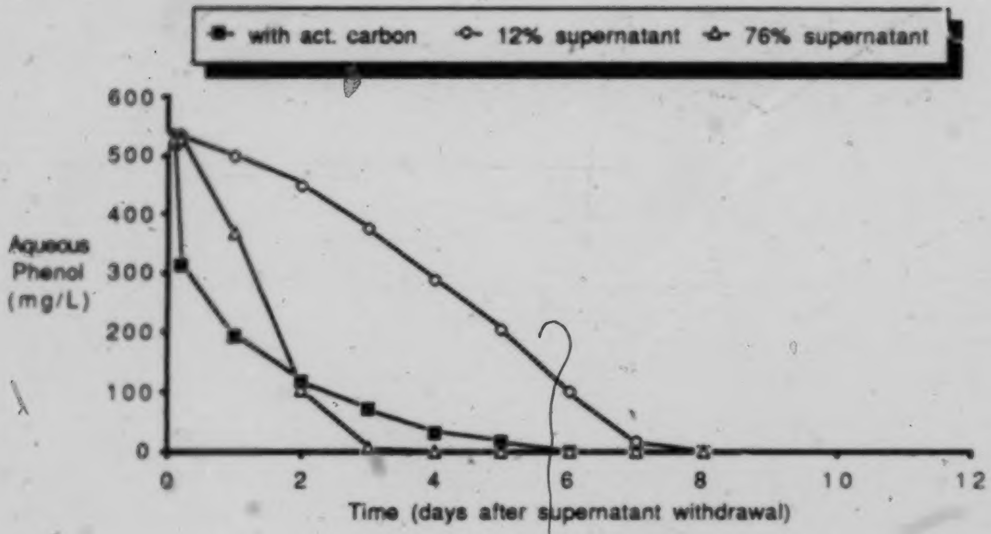


Figure IX.1a Phenol Biodegradation in Culture A with F300 Activated Carbon and in Supernatant Removed From Culture A (1st consecutive supernatant withdrawal)

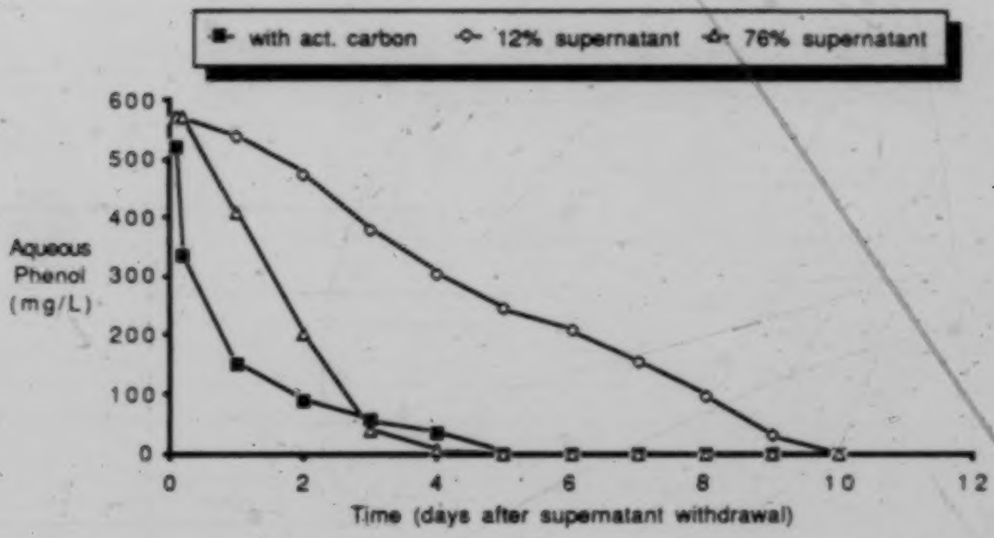


Figure IX.1b Phenol Biodegradation in Culture A with F300 Activated Carbon and in Supernatant Removed From Culture A (2nd consecutive supernatant withdrawal)

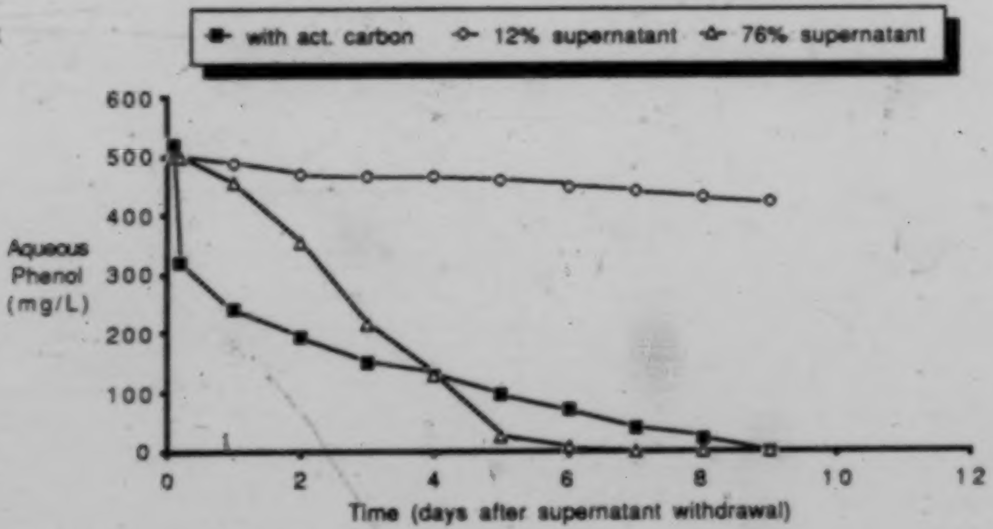


Figure IX.1c Phenol Biodegradation in Culture A with F300 Activated Carbon and in Supernatant Removed From Culture A (3rd consecutive supernatant withdrawal)

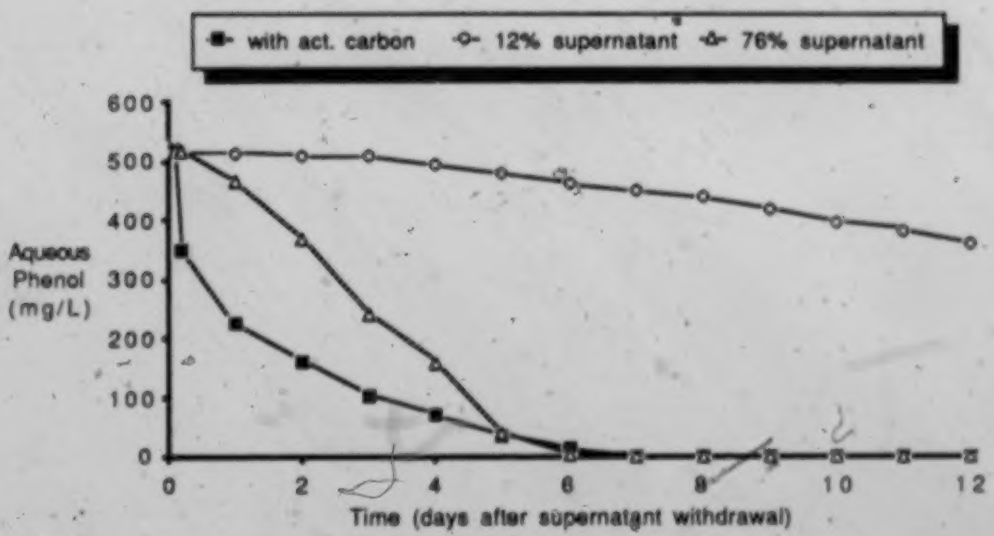


Figure IX.1d Phenol Biodegradation in Culture A with F300 Activated Carbon and in Supernatant Removed From Culture A (4th consecutive supernatant withdrawal)

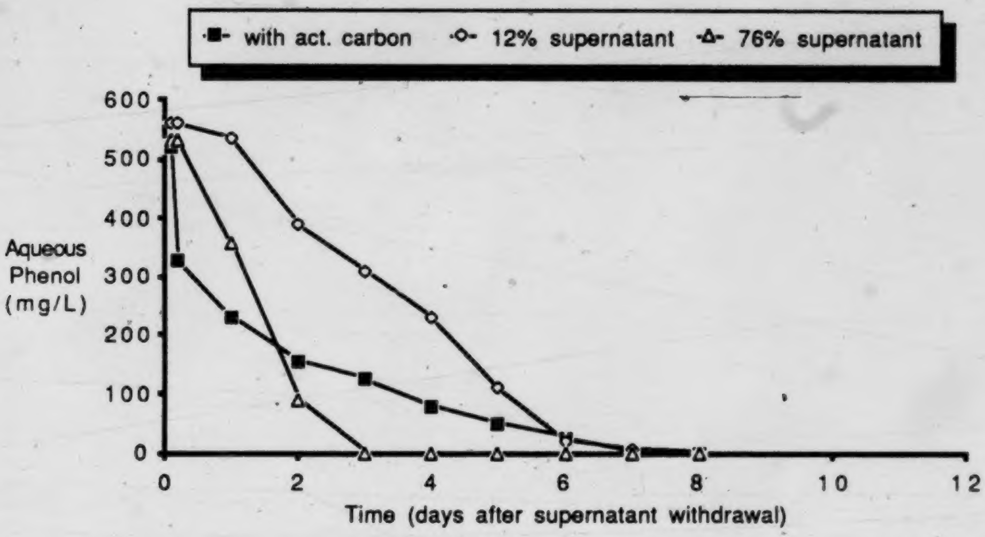


Figure IX.2a Phenol Biodegradation in Culture C with F300 Activated Carbon and in Supernatant Removed From Culture C (1st consecutive supernatant withdrawal)

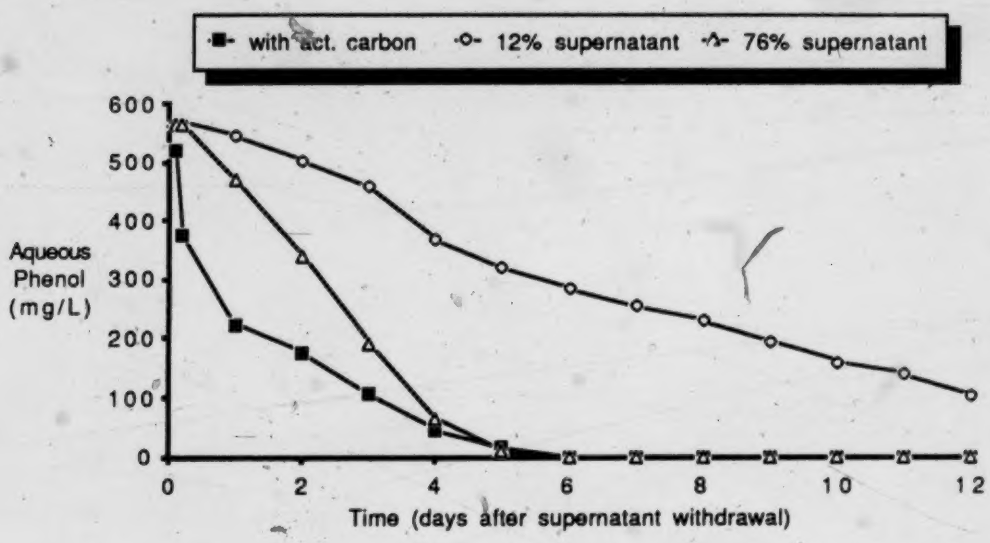


Figure IX.2b Phenol Biodegradation in Culture C with F300 Activated Carbon and in Supernatant Removed From Culture C (2nd consecutive supernatant withdrawal)

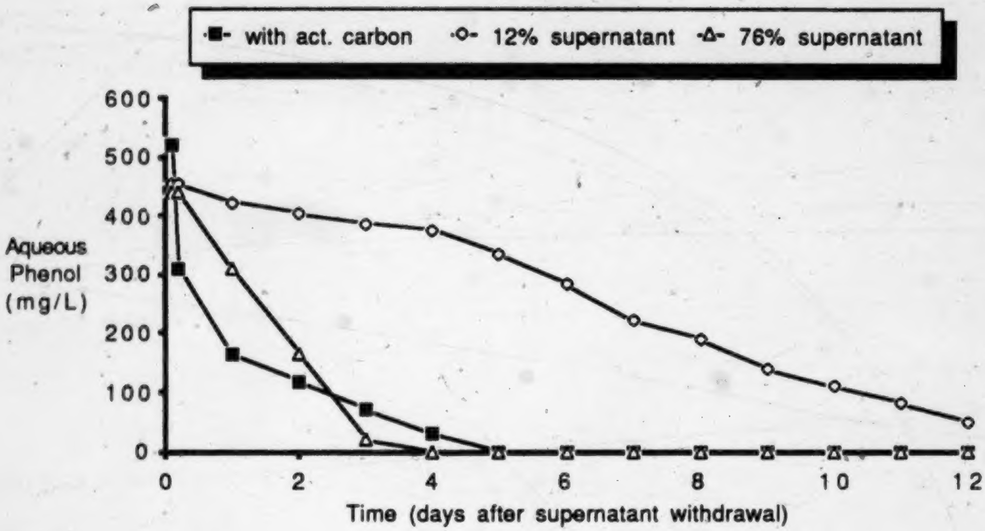


Figure IX.2c Phenol Biodegradation in Culture C with F300 Activated Carbon and in Supernatant Removed From Culture C (3rd consecutive supernatant withdrawal)

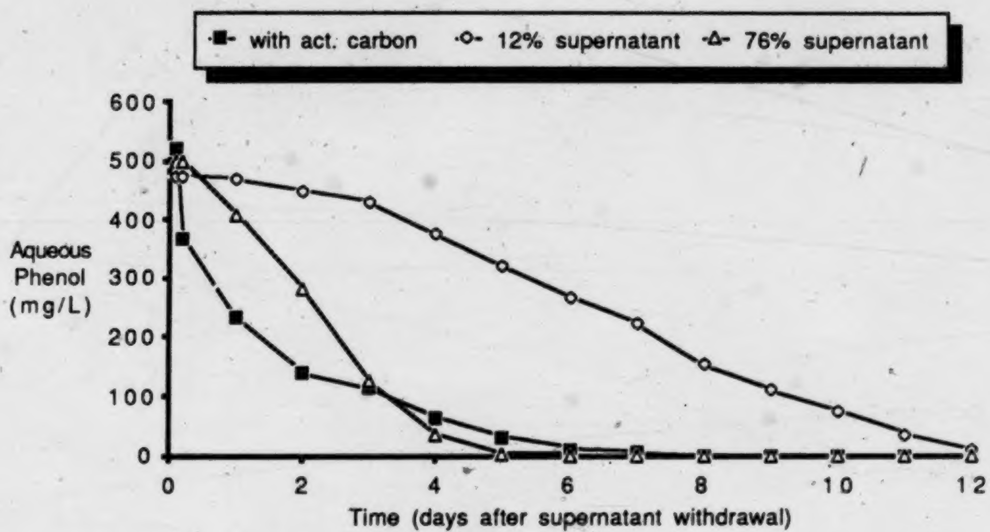


Figure IX.2d Phenol Biodegradation in Culture C with F300 Activated Carbon and in Supernatant Removed From Culture C (4th consecutive supernatant withdrawal)

Appendix X Phenol Biodegradation in Cultures Containing XE-352 Synthetic Ion Exchange Resin

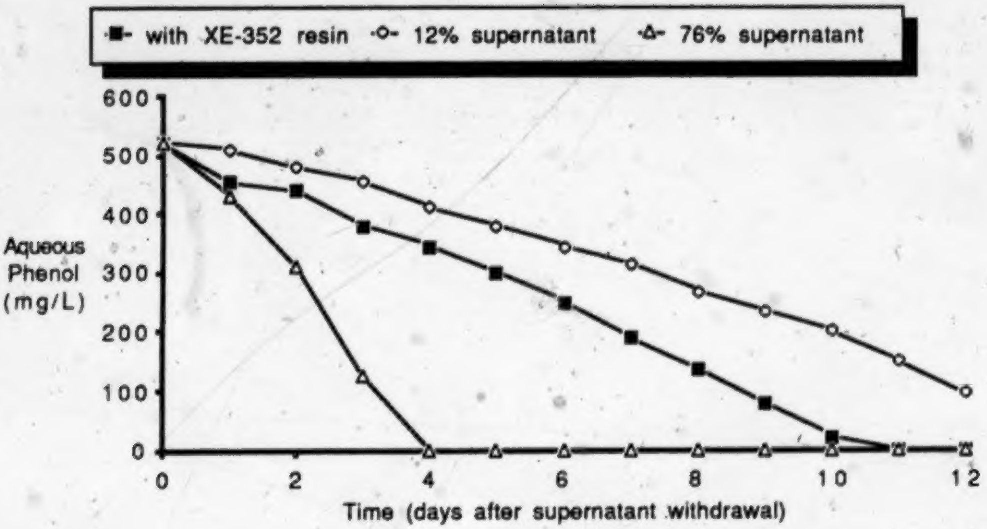


Figure X.1a Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (1st consecutive supernatant withdrawal)

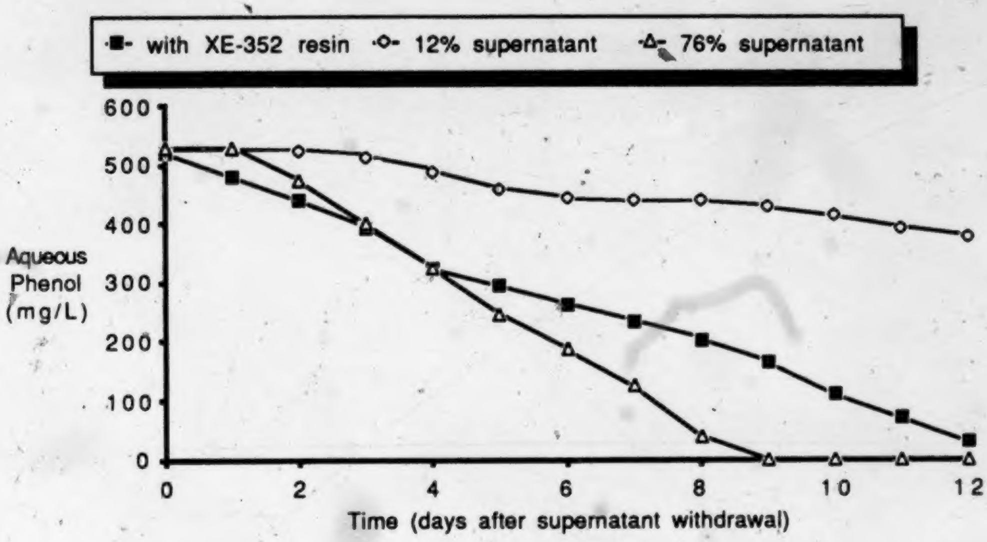


Figure X.1b Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (2nd consecutive supernatant withdrawal)

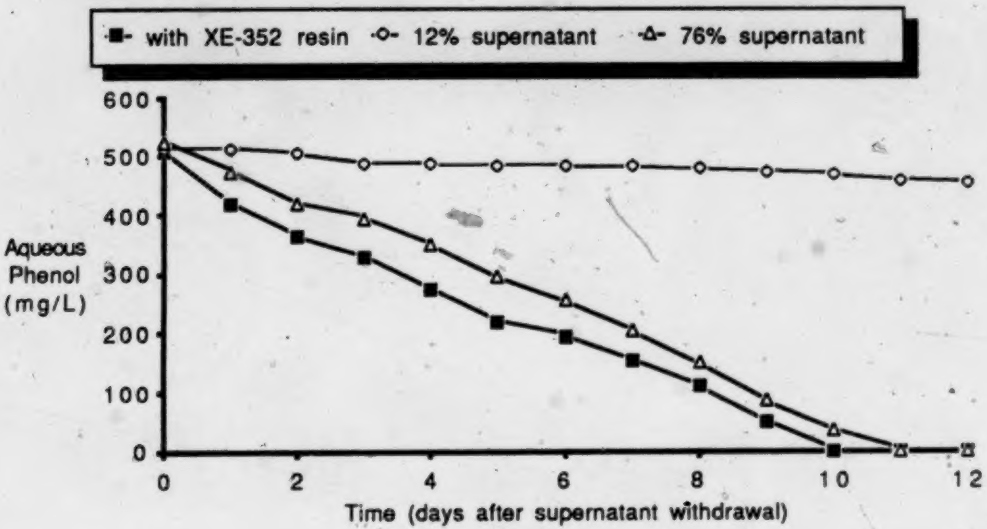


Figure X.1c Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (3rd consecutive supernatant withdrawal)

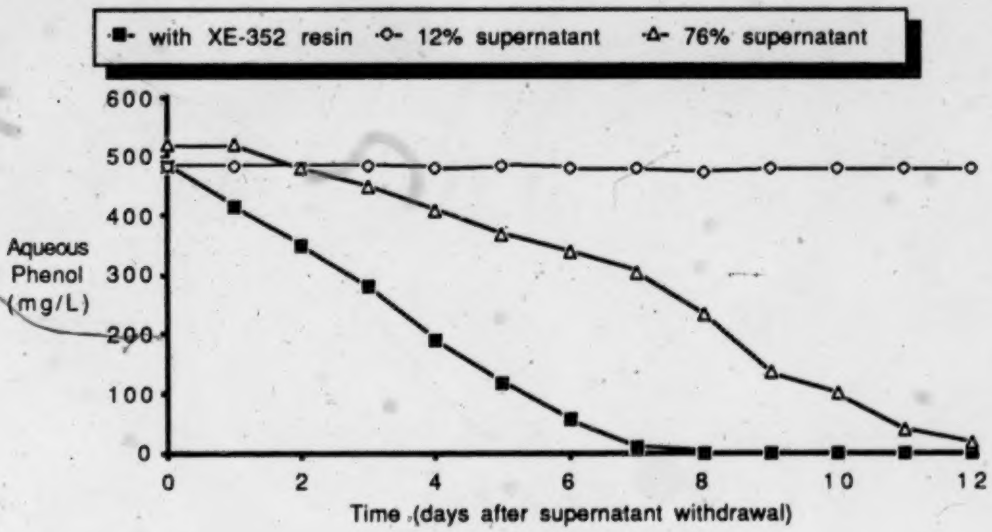


Figure X.1d Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (4th consecutive supernatant withdrawal)

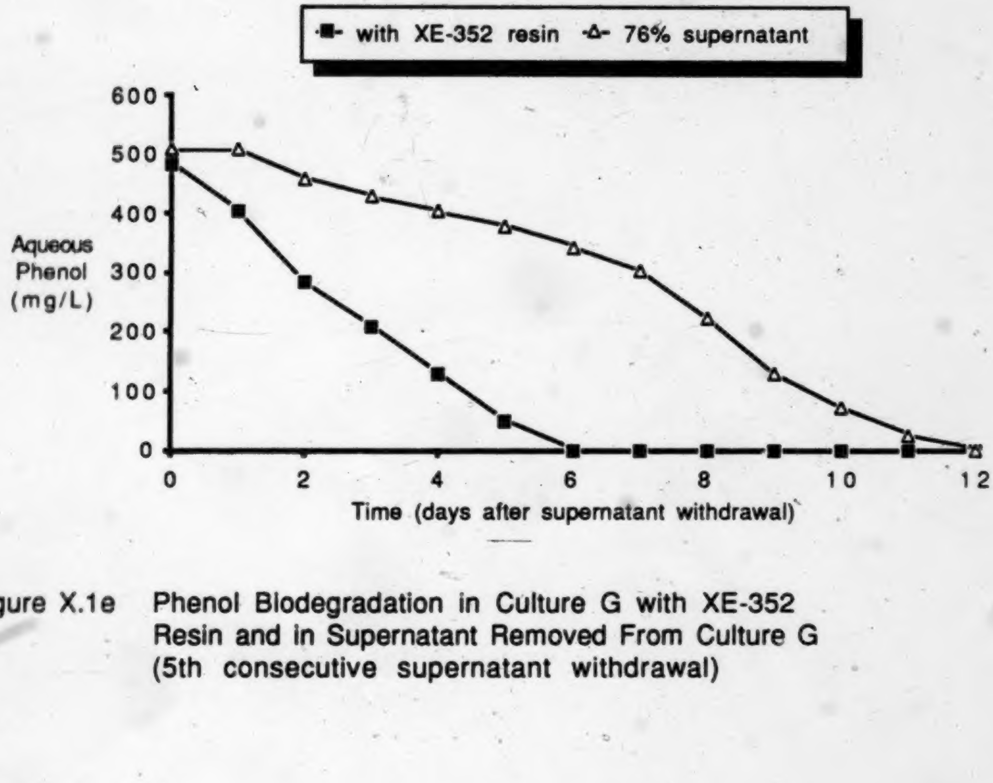


Figure X.1e Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (5th consecutive supernatant withdrawal)

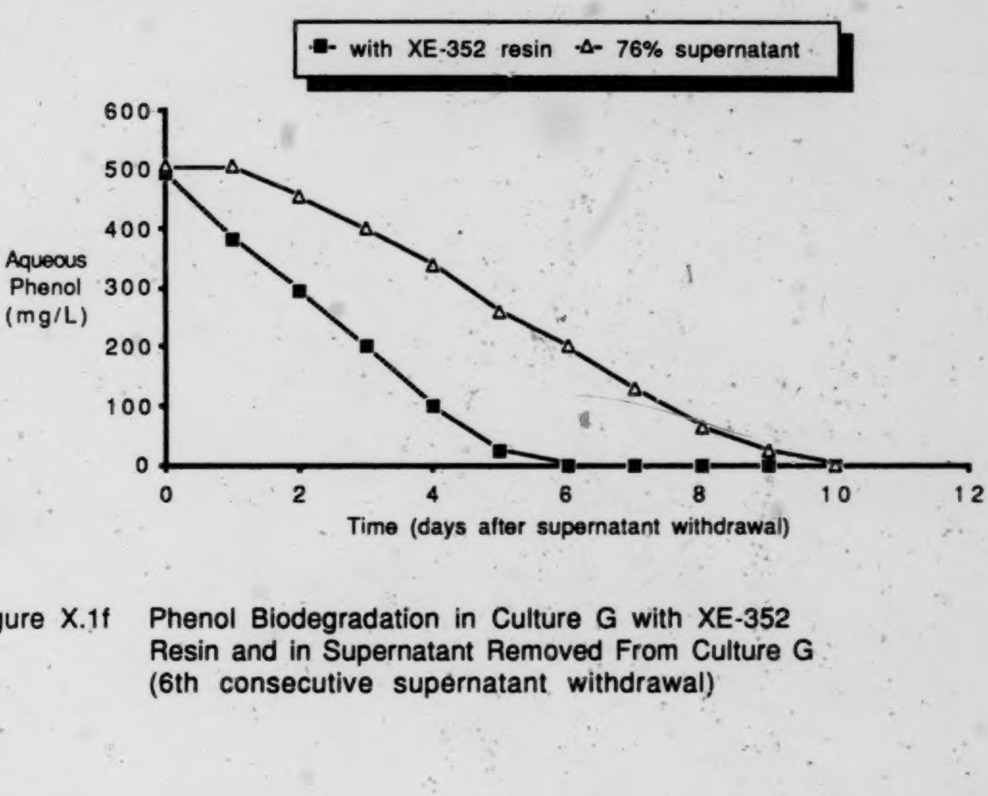


Figure X.1f Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (6th consecutive supernatant withdrawal)

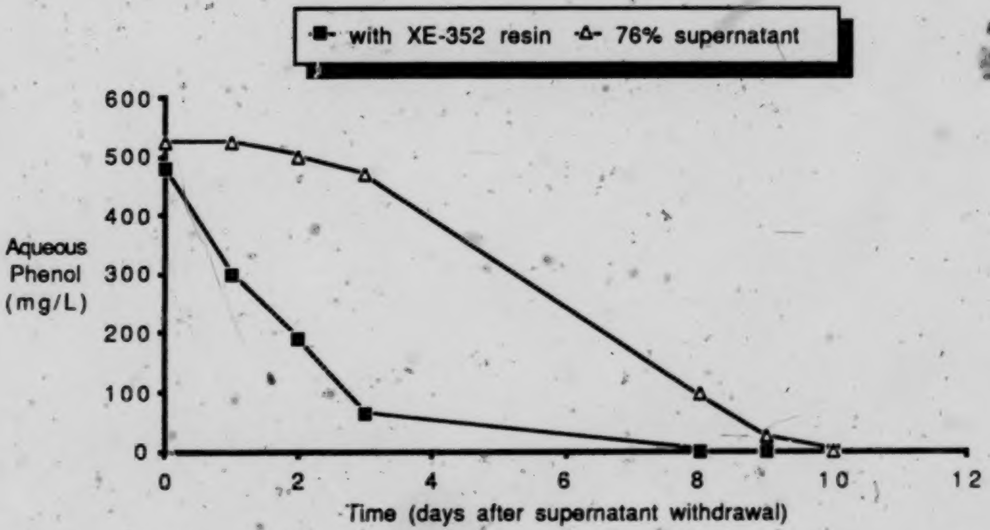


Figure X.1g Phenol Biodegradation in Culture G with XE-352 Resin and in Supernatant Removed From Culture G (8th consecutive supernatant withdrawal)

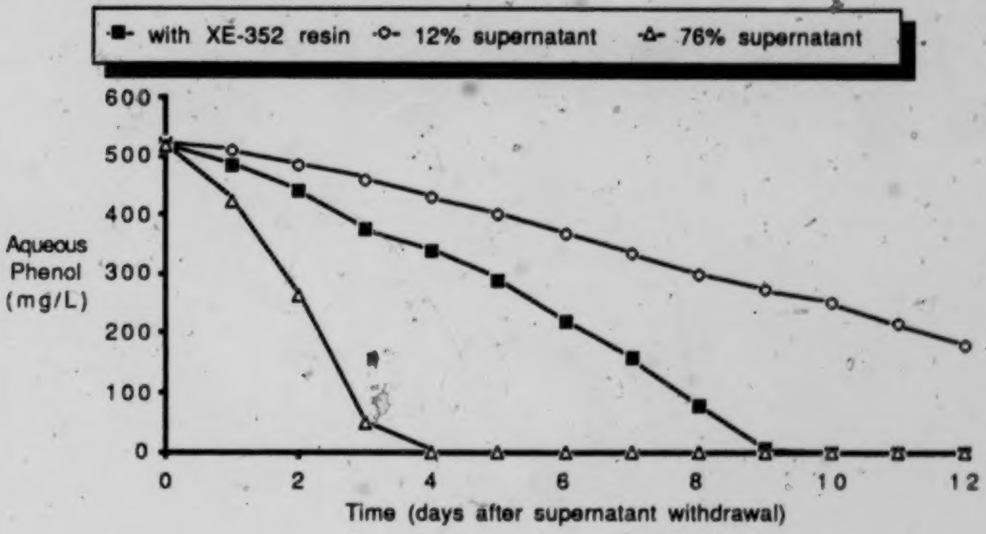


Figure X.2a Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (1st consecutive supernatant withdrawal)

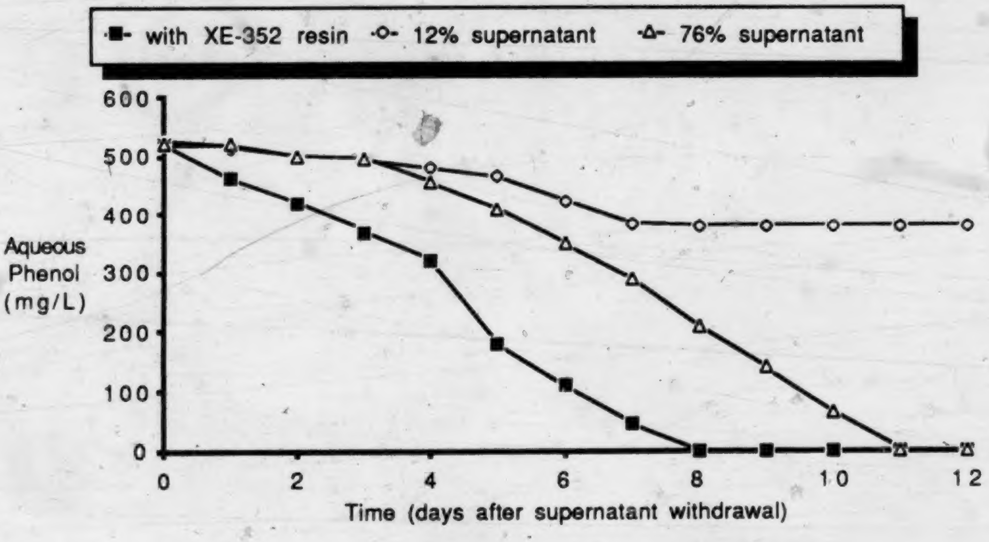


Figure X.2b Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (2nd consecutive supernatant withdrawal)

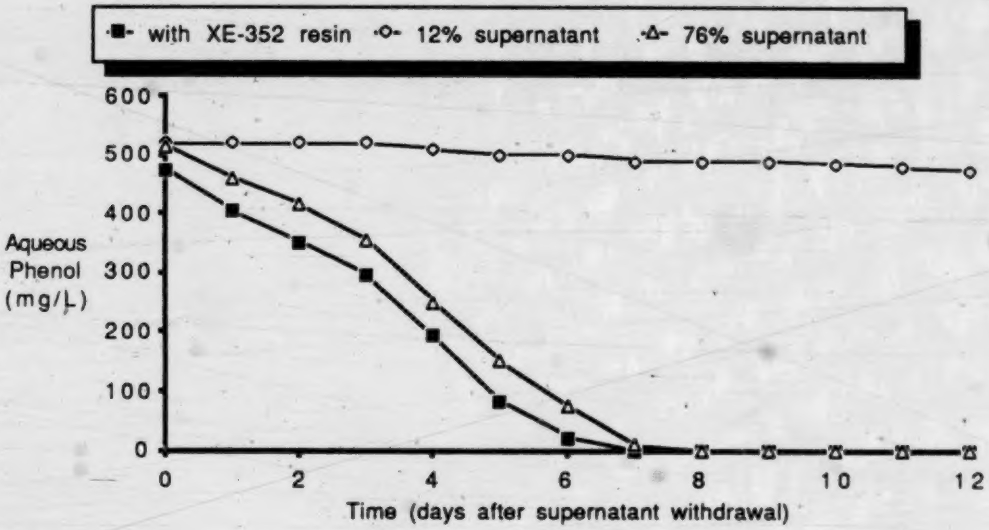


Figure X.2c Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (3rd consecutive supernatant withdrawal)

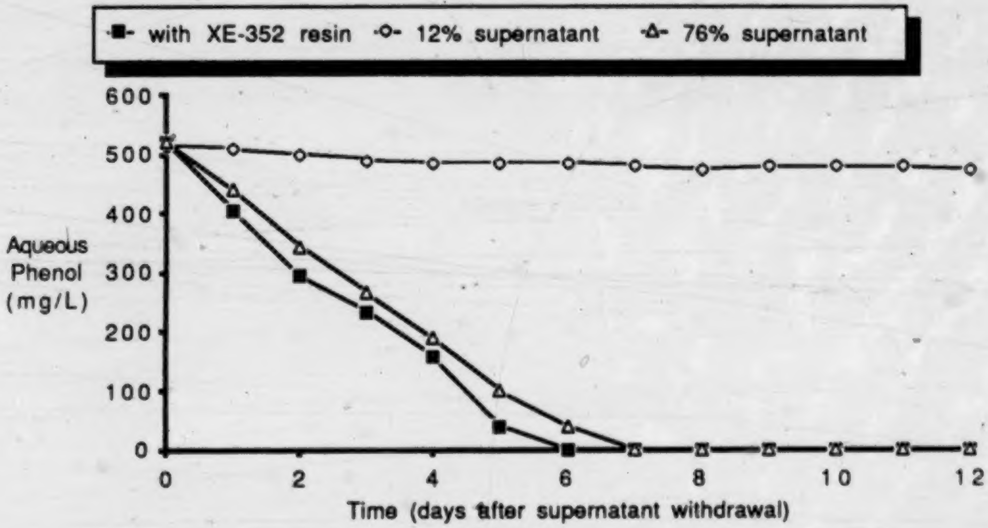


Figure X.2d Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (4th consecutive supernatant withdrawal)

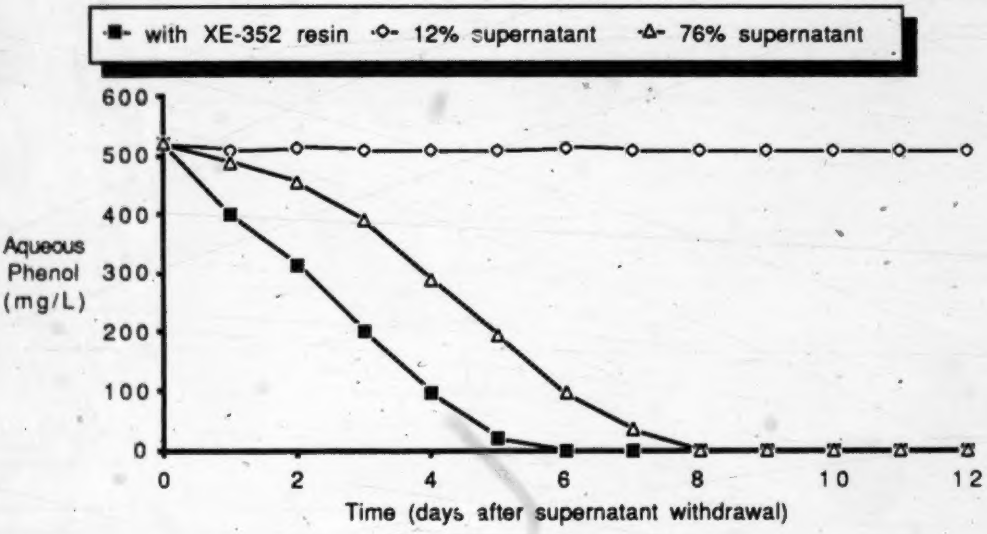


Figure X.2e Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (5th consecutive supernatant withdrawal)

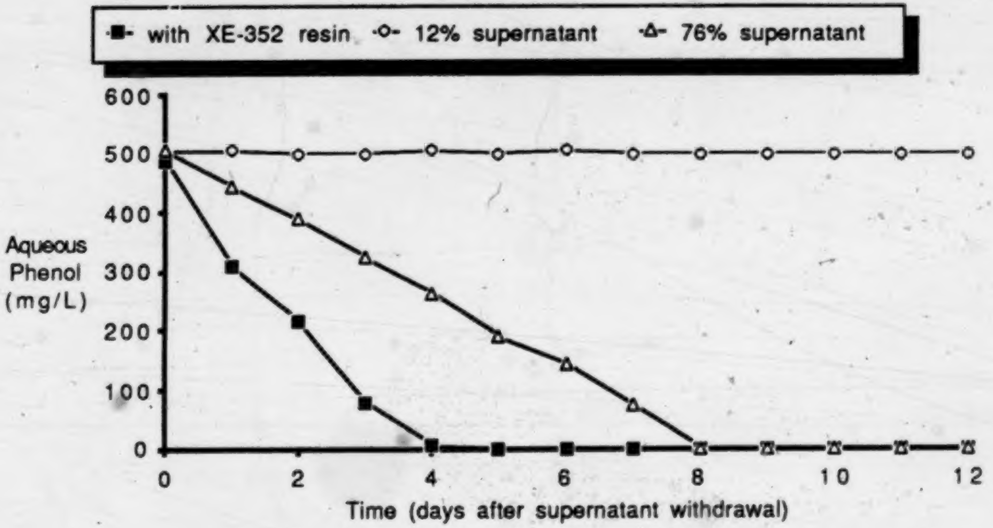


Figure X.2f Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (6th consecutive supernatant withdrawal)

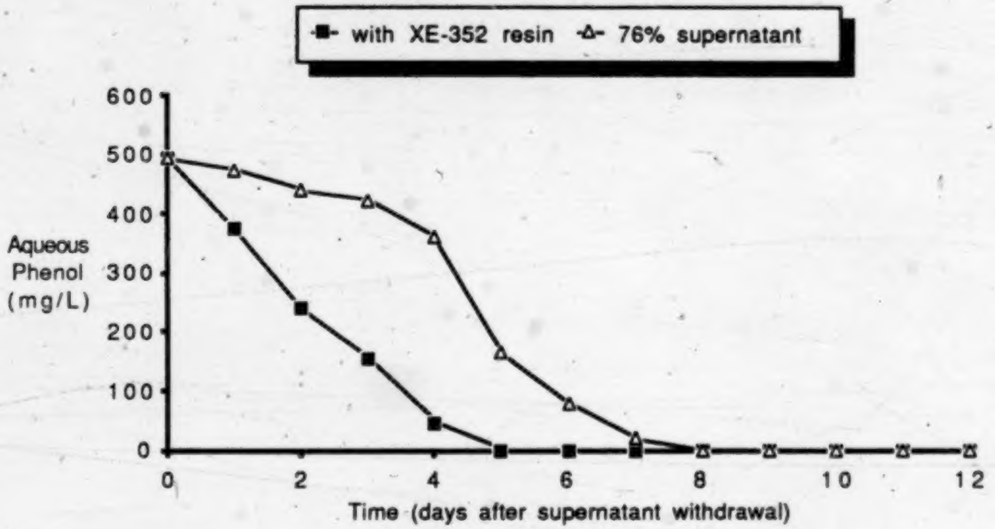


Figure X.2g Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (8th consecutive supernatant withdrawal)

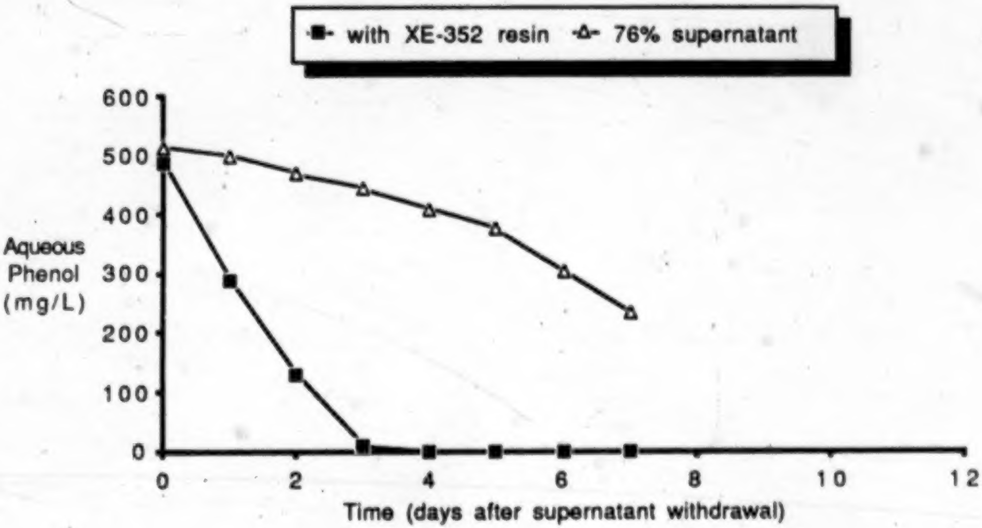


Figure X.2h Phenol Biodegradation in Culture H with XE-352 Resin and in Supernatant Removed From Culture H (10th consecutive supernatant withdrawal)

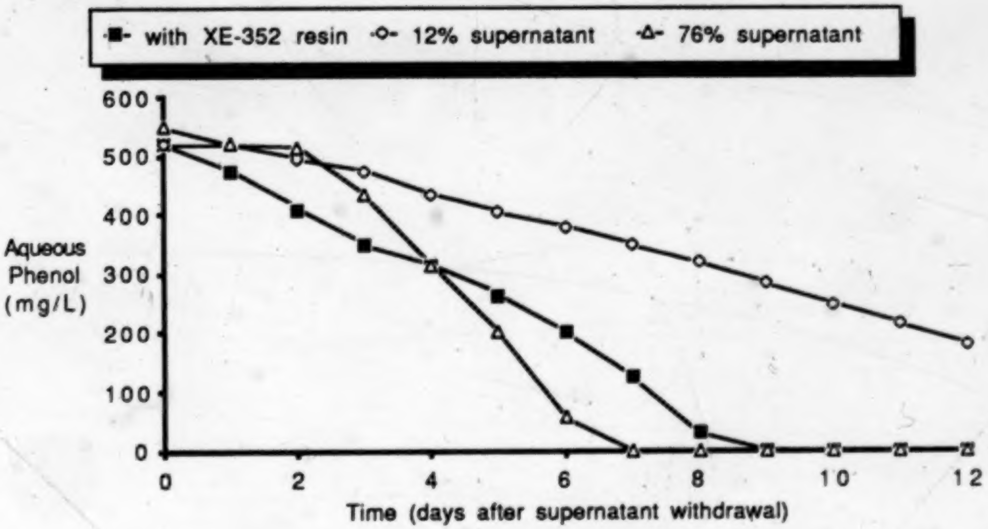


Figure X.3a Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (1st consecutive supernatant withdrawal)

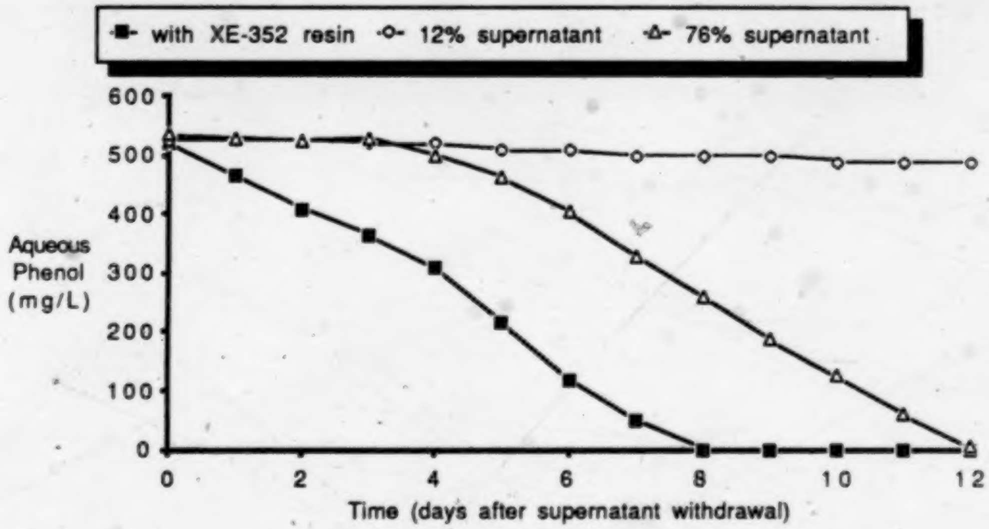


Figure X.3b Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (2nd consecutive supernatant withdrawal)

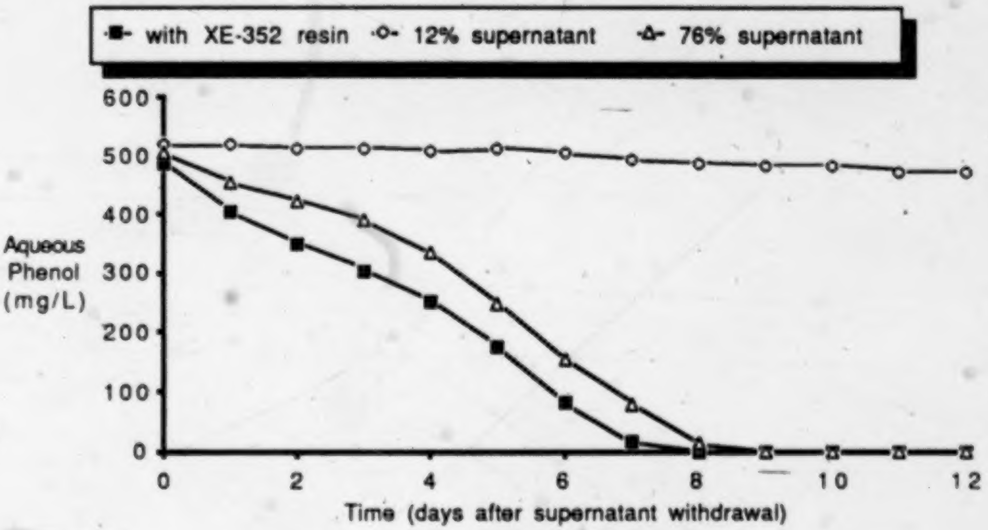


Figure X.3c Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (3rd consecutive supernatant withdrawal)

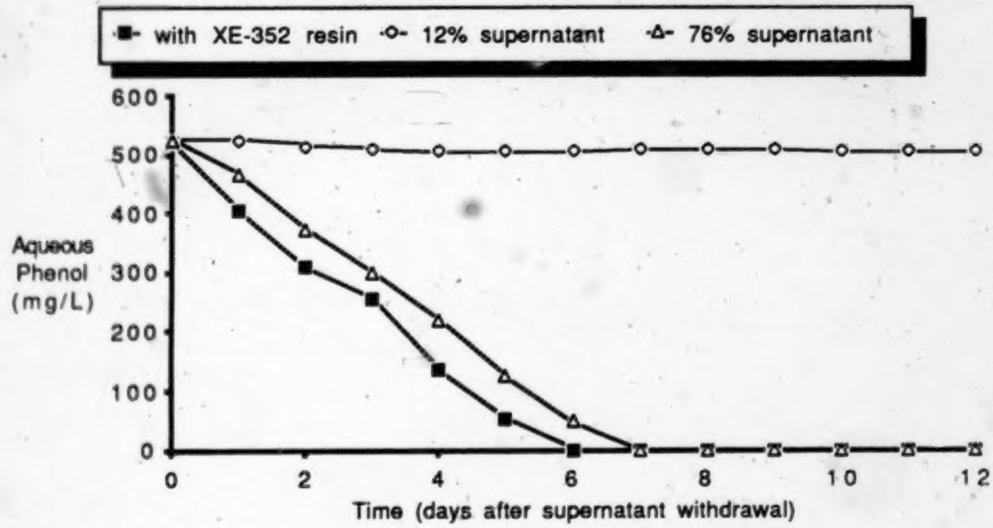


Figure X.3d Phenol Biodegradation in Culture I with XE-352 Resin and in Supernatant Removed From Culture I (4th consecutive supernatant withdrawal)

Appendix XI *Pseudomonas aeruginosa* Adsorption to Support Materials

Table XI.1 *Pseudomonas aeruginosa* Adsorption to F300 Activated Carbon

F300 (mg/mL)	Log [c_e]	Log [q]
16.1	6.50	4.99
33.2	6.32	4.90
49.2	6.16	4.82
65.1	6.05	4.74
81.4	5.85	4.69
97.7	5.70	4.63

c_e = no. of non-adsorbed cells/mL solution after 2 h.
q = no. of adsorbed cells/mg F300 activated carbon.

Note: 2 h contact time was used.
Initial concentration of cells was $10^{6.67}$/mL.

Table XI.2 *Pseudomonas aeruginosa* Adsorption to XE-352 Anion Exchange Resin

XE-352 (mg/mL)	Log [c_e]	Log [q]
2 h contact time:		
0.8	5.51	5.35
2.7	5.25	5.08
4.7	5.03	4.92
7.0	4.88	4.79
8.9	4.85	4.68
11.4	4.67	4.60
4 h contact time:		
1.3	6.56	6.02
3.6	6.30	5.93
5.4	6.11	5.84
8.8	5.91	5.68
11.9	5.75	5.57
15.0	5.65	5.48

c_e = no. of non-adsorbed cells/mL solution after 2 (or 4) h.
q = no. of adsorbed cells/mg F300 activated carbon.

Note: Initial concentrations of cells were $10^{5.70}$/mL for 2 h contact time, and $10^{6.70}$/mL for 4 h contact time.

Table XI.3 *Pseudomonas aeruginosa* Adsorption to MSC-1 Cation Exchange Resin

MSC-1 (mg/mL)	Log [c_e]	Log [q]
18.1	6.68	3.95
38.4	6.68	3.68
59.7	6.68	3.52

c_e = no. of non-adsorbed cells/mL solution after 4 h.
q = no. of adsorbed cells/mg F300 activated carbon.

Note: 4 h contact time was used.
Initial concentration of cells was $10^{6.70}$/mL.